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16. ABSTRACT

The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on nitrate/nitrite. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of nitrate/nitrite
- Toxicokinetics and human exposure to nitrate/nitrite
- Health Effects of nitrate/nitrite in humans and animals
- Mechanisms of toxicity of nitrate/nitrite
- Quantification of toxicological effects of nitrate/nitrite

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I. SUMMARY

Nitrate (the nitrate ion NO_3^-) is the physicochemically stable form of combined nitrogen in oxygenated aqueous systems. Accordingly, there is a tendency for all nitrogenous materials in natural waters to be converted into nitrate. The major point sources for nitrate in drinking water are municipal and industrial wastewaters, septic systems and leachate from animal feed lots and refuse dumps. Diffuse sources include runoff or leachate from manured or fertilized agricultural lands, urban drainage and biochemical nitrogen fixation. Mineral deposits are not a significant source of nitrates in ground waters in the United States.

Orally ingested nitrate ion is not rapidly absorbed from the stomach. Nitrate exits the stomach through the pyloric valve into the small intestine where most of it is absorbed (absorption rate from small intestine unknown, but it is generally assumed to approach 100%). Orally ingested nitrite (the nitrite ion NO_2^-) (reduced nitrate) reacts with many compounds in the stomach contents or is absorbed by diffusion through the gastric mucosa (85% to 100% absorption in ten minutes). Absorbed nitrate circulates in the plasma to all tissues and does not concentrate in any one organ. Absorbed nitrite enters the blood and is rapidly removed by chemical reactions with tissue cells and with hemoglobin.

Nitrate is not metabolized in mammals, but some is secreted into saliva where part of it can be reduced to nitrite by oral bacteria. In humans, about

5% to 10% of ingested nitrate is converted to nitrite by this route. This nitrite is swallowed and in the stomach it either reacts with food or is absorbed along with orally ingested nitrite. In infants, and in adults who have relatively high stomach pH, bacteria can colonize in the stomach and reduce ingested nitrate to nitrite before it reaches the small intestine. This nitrite also either reacts with other stomach contents or is absorbed through the gastric mucosa. Absorbed nitrite in blood oxidizes hemoglobin to methemoglobin which is then reduced by protective enzymes concurrent with oxidation of the nitrite to nitrate.

Nitrate is excreted primarily in the urine (88% recovered within three to four days) and is undetectable in human feces, even after a large single dose. Nitrite is so reactive that it does not reach either urine or feces. Nitrate is excreted in human milk and is reported to have caused methemoglobinemia in a nursing infant.

Nitrate is relatively nontoxic (e.g., lethal toxic doses range from 310 mg/kg in sheep to an LD_{50} in rats of 1,986 mg/kg), but it can become toxic nitrite (e.g., toxic doses range from a lethal dose in fasting rats of 51 mg/kg to an LD_{50} in rats of 120 mg/kg) after bacterial reduction in susceptible species. In species in which absorbed nitrate is not reduced to nitrite, such as the dog, high doses of nitrate have primarily a diuretic effect.

Acute toxic effects of nitrite in animals are manifested as methemoglobinemia with cyanosis and anoxia, and as vasodilation with circulatory collapse. The effect that predominates in a particular species depends on how rapidly nitrite is removed from circulation by the formation of methemoglobin and nitrate. Chronic effects of nitrite include depletion of vitamins A, C and E, and organ pathology reflecting chronic vasodilation and destruction of blood cells.

Nitrate and nitrite have not been found to cause fetal malformation, but nitrite causes delay of maturation of the fetal skeletal system, postnatal growth retardation, and abnormalities of the liver, lungs and spleen in weanling rats. Mutagenicity testing of nitrate and nitrite provided negative results in *in vivo* tests, but nitrite was positive in *in vitro* tests.

Nitrate has not shown any evidence of carcinogenicity. Testing of nitrite for carcinogenicity has been equivocal; two tests reported increases of lymphoreticular tumors in treated rats, but this interpretation has been disputed by other pathologists who reexamined the tissue slides. Two other studies reported increases in liver and stomach tumors in rats, but in both cases the animals were fed diets containing nitrosatable fish meal concurrently with the nitrite. Many studies have documented carcinogenesis, mutagenesis and teratogenesis in animals after oral administration of nitrite and nitrosatable compounds. Tumor sites included (among others) the stomach, esophagus, and nasal cavities. Tumor induction in several of these cases could be inhibited by simultaneous oral administration of ascorbic acid, but in one test, liver tumors decreased and forestomach tumors developed instead. In keeping with established carcinogenicity testing protocol, most doses used were higher than normal exposure.

Adult humans can tolerate large amounts of nitrate in their diet, but nitrite is more toxic. There have been a number of instances where humans have been poisoned by nitrite in food. The nitrite resulted from bacteriological conversion of nitrate to nitrite, from ingestion of food in which the nitrite limit for that food was exceeded and from ingestion as a result of mistaking sodium nitrite for sodium chloride. Symptoms of nitrate or nitrite poisoning in adults are a combination of vasodilatory effects and effects resulting from the formation of methemoglobin.

Severe toxic reactions (including many fatal poisonings) have occurred in infants as the result of ingestion of nitrate at levels which cause no symptoms in adults. The toxicity of nitrate to infants is manifested by methemoglobinemia with resulting cyanosis. This results from bacterial conversion of nitrate to nitrite in the infant's stomach followed by the nitrite's absorption into the blood. In older children and adults, the gastric pH is low enough to inhibit the growth of nitrate-reducing bacteria.

Epidemiological studies have correlated high nitrate intake with cancers of the stomach and esophagus. A lower incidence of these tumors correlated with higher intake of fresh fruits and vegetables, particularly those high in vitamin C, which has been shown to inhibit nitrosation by nitrite in animals.

The presence of nitrate in water is frequently associated with other contaminants, notably bacteria and nitrites. Microbiological contamination is already regulated, but nitrite has traditionally been omitted from drinking water regulations. It has been assumed that the reducing conditions necessary for the preservation of the nitrite species are uncharacteristic of properly maintained public drinking water supplies and that water sufficiently polluted to foster the presence of nitrites would exceed the microbiological contaminant levels. This dependence on microbiological limits may not be adequate because nitrites are detected in water which does meet the U.S. Environmental Protection Agency coliform limits.

Nitrite is more hazardous to health than nitrate, in terms of both methemoglobinemia and potential carcinogenicity. In addition, the presence of significant concentrations of nitrite in drinking water is an indication of gross contamination of the water source. The presence of significant concentrations of nitrate can also be deemed an indication of contamination.

A No-Observed-Adverse-Effect Level (NOAEL) of 111 mg/L for children exposed to nitrate-nitrogen in drinking water was used to establish ten-day Health Advisories (HAs) for adults and all age groups other than the infant for both nitrate- and nitrite-nitrogen. The HA values are 111 and 11 mg/L for nitrate- and nitrite-nitrogen, respectively. A NOAEL of 10 mg/L for infants was used to establish a ten-day HA for infants of 10 mg/L for nitrate-nitrogen and 1 mg/L for nitrite-nitrogen. Due to the absence of suitable data, and the fact that the effects of methemoglobinemia are rapidly reversible and result in no accumulative effects, it is appropriate to use the ten-day HA values for one-day exposures also. Adjusted Acceptable Daily Intakes (AADIs) of 10 mg/L and 1 mg/L for nitrate- and nitrite-nitrogen, respectively, were calculated from a NOAEL of 10 mg/kg/day nitrite-nitrogen for infants exposed to nitrate-nitrogen in drinking water.

The current United States drinking water standard is 10 mg/L nitrate-nitrogen. This is based entirely on reports of infant methemoglobinemia resulting from levels above the standard. Additional data indicate that nitrate and nitrite from all sources increase the risk of formation of mutagenic and carcinogenic N-nitroso compounds in the human stomach, especially in persons with high exposure to nitrosatable compounds in drugs, pesticides and industrial air pollution.

II. PHYSICAL AND CHEMICAL PROPERTIES

A. Physical and Chemical Properties

Properties of nitrate and nitrite are summarized in Table II-1. Nitrate (the nitrate ion NO_3^-) is the physicochemically stable form of combined nitrogen for oxygenated aqueous systems. The nitrate salts of all common metals are quite soluble in water. Nitrate has little tendency to form coordination complexes with metal ions in dilute aqueous solution. In dilute aqueous solutions, nitrate is chemically unreactive, and nearly all of the transformations involving nitrate in natural waters are mediated biochemically. Nitrate can be reduced by microbial action, and many of the deleterious effects of nitrate in the environment result from its conversion to nitrite (the nitrite ion NO_2^-) (NAS 1978).

Nitrite ion contains nitrogen in an intermediate and relatively unstable oxidation state. Both chemical and biological processes can result in the further reduction of nitrite to various compounds or in oxidation back to nitrate. Enzymatic processes in certain bacterial, fungal and plant systems can reduce nitrate and both oxidize and reduce nitrite as part of the natural nitrogen cycle (NAS 1978).

Nitrite ion is the conjugate base of a weak acid, nitrous acid (HNO_2). In most natural waters, the acid is dissociated into ions, but in acidic environments, a significant fraction can exist as the undissociated species. Of the many reactions involving nitrous acid, the reaction with amino

Table II-1 Properties of Nitrate and Nitrite

	Nitrate	Nitrite
Formula	NO_3^-	NO_2^-
Usual state	Ion in aqueous solution at neutral pH	Ion in aqueous solution at neutral pH
Acid	Conjugate base of strong acid HNO_3, pKa = -1.3	Conjugate base of weak acid HNO_2, pKa = 3.4
Salts	Nearly all soluble in water, dissociated at neutral pH	Forms salts with Li Na K Ca Sr Ba Ag
Reactivity	Unreactive	Reactive Oxidizes antioxidants Oxidizes Fe^{++} of hemoglobin to Fe^{+++} Oxidizes primary amines Nitrosates secondary amines, tertiary amines, ureas, carbamates, guanidines

Adapted from NAS 1978, 1981.

substrates to form N-nitroso compounds is presently a great environmental and public health concern because many N-nitroso compounds are carcinogenic (NAS 1978).

Nitrosation in aqueous nitrous acid at pH<5 proceeds by reaction of the unprotonated amino substrate with an electrophilic nitrosating agent such as dinitrogen trioxide (N_2O_3) or the hydrated nitrosonium ion (H_2O^+-NO). Basic secondary amines react most rapidly at pH 3.4, but these reactions are generally slower than those for amides, ureas and carbamates (NAS 1981).

Nitrosation of secondary amines by aqueous nitrous acid is accelerated by nucleophilic anions such as thiocyanate and iodide. Comparable nitrosation reactions of amides, ureas, guanidines and carbamates are not usually accelerated by these catalysts (NAS 1981).

Nitrosation of secondary amines, amides, ureas and guanidines by aqueous nitrous acid can be inhibited by compounds that reduce nitrous acid to nitric oxide. These inhibitors include ascorbic acid (vitamin C), α-tocopherol (vitamin E) and several naturally occurring polyphenolic antioxidants (NAS 1981).

The chemical reactions for nitrite forming N-nitroso compounds and the role of hydrogen ion in these reactions are explained in detail in Section VII.

B. Manufacture and Uses

The major use of nitrate is in inorganic fertilizers. A production summary of the principal inorganic nitrogen fertilizers for August 1981, as reported by the U.S. Bureau of the Census, in short tons (2,000 lbs) is as follows: ammonia - 1,510,000; ammonium nitrate - 617,000; nitric acid - 657,000 (USDC 1981).

Other uses for which no quantitative data were found include the production of explosives and the production of purified potassium nitrate for glassmaking. A blend of nitrate salts has been developed recently for applications in central receivers for solar heat (Anon 1981b). The new product is called solar nitrate salt and is used as a heat-transfer fluid and a heat-storage medium.

Sodium nitrite in food grade is being produced for use as a preservative in cured meats (Anon 1981a). Nearly four billion kilograms of cured meat products were processed with added nitrite in the United States during 1979. The use of nitrate in food products has been declining but it is still added to a variety of products as a reservoir for nitrite; nitrite is quite unstable in meat and is rapidly lost - if bacteria are present, they will reduce nitrate to nitrite and inhibit their own germination and growth (NAS 1981).

C. Summary

Nitrate is a relatively stable component of the natural environment and is found in all vegetated areas. It is normally at insignificant concentration in water, but can reach health-threatening levels as a result of agricultural runoff, refuse dump runoff, or contamination with human or livestock wastes. Nitrate is manufactured primarily for use as fertilizer, and this contributes to the contamination of water supplies.

Nitrite is an unstable and reactive intermediate in the nitrogen cycle. It is manufactured for use in curing meats and normally does not contaminate drinking water.

III. TOXICOKINETICS

A. Absorption

Witter et al. (1979b) administered oral radioactive nitrate ion ($^{13}NO_3^-$) (dose not reported) to two male volunteers and followed the radioactive label with a computer-linked Auger camera. One subject received nitrate orally about an hour after a large meal. The half-time for the disappearance of the label from the stomach was about 30 minutes. The radioactivity in the pylorus remained almost constant, suggesting that nitrate is not rapidly absorbed from the stomach but exits into the small intestine. The second subject was given nitrate about ten hours after eating. The half-time for the disappearance of the label from the stomach of the second subject was less than ten minutes.

A similar study was performed (doses not reported) with Sprague-Dawley rats of undisclosed sex, some of which were previously ligated at the pyloric valve (Witter et al. 1979a). The group with pyloric ligation retained nearly all of the radioactivity in the stomach 40 to 50 minutes after administration. Only 21% of the radioactivity was retained in the rats without pyloric ligation, suggesting that nitrate leaves the rat stomach predominantly through the pyloric valve and is not absorbed from the stomach. In the rats which were not ligated, essentially all of the radioactivity passed into the upper small intestine, where most was absorbed. Similar studies using $^{13}NO_2^-$ resulted in some gastric absorption, but most of the radioactivity seemed to exit the stomach via passage into the duodenum. The exit of nitrite from the stomach was slower, and less radioactivity appeared to be absorbed from the small intestine than with nitrate.

Witter and Balish (1979) studied oral absorption of nitrate and nitrite ions using a spectrophotometric assay to differentiate the ions from their metabolites. Sprague-Dawley rats (60- to 90-days-old, sex not reported) were fed the ions in their drinking water at 1 mg/mL (160 mg/kg/day) for an unreported period. Various parts of the intestinal tract were removed and analyzed for nitrate and nitrite ion content. Rats fed nitrate had both nitrate and nitrite in the stomach, but only nitrate in the small intestine. Rats fed nitrite had both ions in the stomach and small intestine. Control rats had trace amounts of only nitrate in their stomachs. These results are in agreement with the above results (Witter et al. 1979a, 1979b), suggesting that nitrate is absorbed from the upper small intestine and nitrite from the stomach. However, when a large dose of nitrite is administered, some passes into the intestine.

Friedman et al. (1972) performed a colorimetric assay of nitrite absorption in male Swiss ICR/Ha mice weighing between 20 and 25 grams. Mice were given food and water ad lib while 150 μg of sodium nitrite (6.8 mg/kg) was administered to each mouse by gavage. Groups of 13 to 18 mice were sacrificed within one minute and at 10, 20 and 30 minutes after sodium nitrite administration, their stomachs were removed and assayed. At ten minutes after oral administration, 85% of the available sodium nitrite had disappeared from the mouse stomach. Ligation of the gastroduodenal junction had no effect on nitrite loss. Other experiments were performed using isolated mouse stomachs where loss of nitrite by absorption was prevented. After a 30-minute incubation, there was 63% loss of nitrite, of which 40% had been converted to nitrate. The authors concluded that the major pathway for disappearance of available gastric nitrite is absorption directly from the stomach into the bloodstream. The rate of disappearance of nitrite from the mouse stomach fits second-order kinetics. This is consistent with the fact that in acid conditions, nitrite forms an uncharged compound, N_2O_3. Uncharged molecules generally pass through membranes more rapidly than charged molecules, so the uncharged N_2O_3 is probably the form absorbed.

Parks et al. (1981) studied the absorption of $^{13}NO_3^-$ and $^{13}NO_2^-$ after intratracheal instillation of 10 to 100 ng/kg (0.00001 to 0.0001 mg/kg) in groups of 10 to 12 Balb/C mice. In this study 100% of the label was absorbed within ten minutes.

No information on dermal absorption of nitrate or nitrite was found in the literature.

B. Tissue Distribution

Witter et al. (1979b) gavaged nine rats (sex and strain not reported) with $^{13}NO_3^-$ (dose not reported) and 45 to 60 minutes later organs were removed and the radioactivity measured. The largest fraction, 47.3%, was found in the carcass after removal of abdominal organs. This was more than could be accounted for as blood-bound activity. To study the fate of bloodstream nitrate, the same authors injected $^{13}NO_3^-$ intravenously into nine rats of which one was ligated at the pyloric junction and three at the ileocecal junction. Except for the stomach, which contained 5.6% to 6.8% of the radioactivity, no marked accumulation was seen in any organ at 50 minutes and the radioactivity was mainly in the eviscerated carcass (74.2% to 78.8%). Thirty minutes after injection, the intestinal tract from the duodenum to the large intestine contained essentially the same amount of radioactivity even if the pylorus was ligated. This indicated that the label of $^{13}NO_3^-$ could enter the intestines from the blood, either as part of the biliary or pancreatic secretions or by direct secretion from the bloodstream. Data from rats ligated at the ileocecal junction suggested secretion directly into the lower intestines from the blood.

Witter et al. (1979b) also injected $^{13}NO_3^-$ intravenously into a human volunteer and followed the radioactivity with an Auger camera. There was rapid distribution of the label. The radioactivity in the heart region reached a maximum of about 3% of the administered dose at two minutes, as it passed through the heart for the first time. The activity then fell rapidly in the next two minutes as the radioactive label became evenly distributed in the blood. The radioactivity accumulated almost linearly with time in a fist-sized region of the abdomen, probably due to the swallowing of salivary $^{13}NO_3^-$. The remainder was fairly evenly distributed throughout the body, with less than 5% measured in the urine or salivary glands within 40 minutes. The salivary glands of intravenously injected rats accumulated activity to only one-tenth of that seen in the human mouth.

Parks et al. (1981) studied the distribution of $^{13}NO_3^-$ and $^{13}NO_2^-$ after intratracheal instillation in Balb/C mice and intravenous injection in mice and New Zealand White rabbits. The protocol for this study was presented in Section IV.A. Mice were sacrificed from 5 to 30 minutes after administration of the labeled ions and organs were excised and weighed. Tracer concentrations were determined by gamma-ray counting. Both tracers achieved transient equilibrium very rapidly for each route of administration. The distribution data were relatively constant within five minutes. The specific activities did not differ significantly between organs or routes of administration. The authors concluded that either the ions distribute equally into the same body space or they chemically transform into each other or into similar products.

The rabbits injected intravenously were studied with the Auger scintillation camera and by blood analysis. A rapid homogeneous distribution of the radioactivity throughout the rabbit was observed. Equilibrium between the intravascular and extravascular compartments was reached within five minutes after injection of either radiochemical. Only 2% to 3% of the label appeared in the bladder during the first 30 to 45 minutes after injection (Parks et al. 1981).

Portions of each supernatant from the blood distribution experiments in mice and rabbits were analyzed by high-pressure liquid chromatography (HPLC). The chromatographic results obtained from mouse plasma taken ten minutes after intratracheal instillation of labeled nitrite revealed that 70% had been converted to nitrate, 27% remained nitrite and about 3% was in nonionic compounds. The product distribution in rabbit plasma ten minutes after intravenous injection of labeled nitrite was 51% nitrate, 46% nitrite and 3% in nonanionic compounds. Ten minutes after administering labeled nitrate to both species, the label found in blood remained nitrate. Any nitrite formed by the bacterial reduction of nitrate was not detectable (Parks et al. 1981).

Edwards et al. (1954) studied salivary ions in normal people and patients undergoing treatment with radio-iodine ($^{131}I^-$). Numbers and doses were not reported. Anions were given as oral doses of the potassium salt and thiocyanate was detected by chemical assays. They concluded that there is antagonism between iodide, thiocyanate, perchlorate, and nitrate for secretion in saliva. This suggests a common receptor for active transport into saliva. Nitrate was less effective than thiocyanate or perchlorate in depressing salivary secretion of iodide, and less effective than iodide or perchlorate in depressing salivary secretion of thiocyanate.

Spiegelhalder et al. (1976) used a colorimetric assay to study secretion of nitrate in saliva of 11 volunteers after ingestion of vegetables and vegetable juices. Nitrate concentration was determined at intervals of 30 or 60 minutes for seven hours. The increase in the amount of nitrate secreted by the salivary glands was found to depend directly on the amount of nitrate ingested above a threshold dose of 54 mg. About 20% to 25% of ingested nitrate is secreted. The concentration starts to rise 30 minutes after nitrate ingestion and peaks from one to three hours later depending on the food or juice consumed. Swallowed salivary nitrate recirculates through the blood to the saliva again, causing one or more additional smaller peaks of secretion.

Secretion of nitrate by the gastric mucosa was studied by Bloomfield et al. (1962) in pylorus-ligated rats of unreported strain. Doses of sodium nitrate ranging from 60 to 200 mg/kg were injected intraperitoneally into fasted rats. As the plasma concentration increased, the gastric juice concentration also increased. The gastric juice to plasma ratio dropped from a value of 20 when the nitrate concentration in plasma was 1 mM to unity at 4.5 mM. This suggests that an active transport mechanism is involved and it can be saturated. When nitrate and $^{131}I^-$ were injected simultaneously, there was less radioactivity in the gastric juice than when $^{131}I^-$ was injected alone. This suggests that nitrate and iodide have a common concentrating mechanism in the rat stomach.

C. Metabolism

Parks et al. (1981) found no evidence of mammalian ability to metabolize nitrate into other compounds. Their experimental protocols were presented in Section III.B.

1. Reduction of Nitrate in Saliva

Nitrate can be reduced to nitrite and then to ammonia and other products by bacteria. The reduction of nitrate in saliva by human oral bacteria was studied by Goaz and Biswell (1961). Saliva was obtained by paraffin stimulation, collected in test tubes immersed in crushed ice, and used in less than an hour. Nitrate and nitrite concentrations were determined colorimetrically. When whole saliva containing 28 mg/L nitrate was incubated at 37°C, nitrate concentration decreased rapidly and was not detectable after 75 minutes. Nitrite accumulated transiently, increasing to 5 mg/L at 45 minutes and then decreasing to zero by 105 minutes of incubation. Saliva depleted of its endogenous nitrate and nitrite by prior incubation will rapidly reduce added nitrate. Further experiments determined that nitrate reduction to nitrite in human saliva occurs under either aerobic or anaerobic conditions, but the presence of oxygen completely inhibits the reduction of nitrite. Maximum activity occurred between pH 6 and 6.4, with no reduction of nitrate below pH 4 or above pH 9. Between pH 8.2 and 8.6 nitrate is reduced to nitrite but nitrite reduction is inhibited.

Eisenbrand et al. (1980) studied salivary nitrate reduction using 11 volunteers who had ingested varying amounts of nitrate in red beet juice or spinach. Using a colorimetric assay, they showed a direct correlation between the amounts of nitrite produced in saliva and the amounts of nitrate ingested. About 20% of the secreted nitrate was converted to nitrite. The same authors determined the mean and range of nitrite concentrations in saliva of different age groups in Germany. There was a steady increase from a mean of 0.6 mg/L at one week to six months of age, up to a mean of 6.8 mg/L in adults.

2. Reduction of Nitrate in the Stomach

Franklin and Skoryna (1971) studied the survival of microorganisms in the gastric lumen of fasting human subjects. The subjects were 13 hospitalized adults having sterilized nasogastric tubes in place. Sterile samples of gastric fluid were taken hourly and cultured. It was concluded that the survival of bacteria in the gastric lumen depends on the pH of the gastric juice. At pH 4 or higher, all samples showed growth of microorganisms and the percent of samples with few or no organisms increased with decreasing pH. When a rapid drop in pH occurred in a patient previously measured at pH greater than 4, the clearing of viable organisms occurred within one hour in most cases. The pH of a normal adult human stomach is less than 3.

Lactobacilli in breast-fed infants do not reduce nitrate to nitrite and maintain a pH low enough to discourage other bacteria from colonizing the infant's stomach. Marriott and Davidson (1923) determined the normal acidity of infants' stomachs (under one-year-old) using four-color pH indicators to assay samples of gastric contents. Samples were withdrawn through a rubber catheter two hours after feeding different diets. Three mL of gastric contents were dialyzed against 3 mL of sterile physiologic saline for 15 minutes. The dialysate was assayed for pH and compared with the standard buffer solution. The gastric pH of 23 breast-fed normal infants averaged 3.75. When these infants were fed undiluted cow's milk, which has a high buffering capacity, their average gastric pH rose to 5.3. This is high enough to allow heavy bacterial growth. In another group of infants accustomed to cow's milk, the average pH following a meal of undiluted whole cow's milk was 4.75, indicating an adaptation to this type of food. A group of 16 infants aged three weeks to nine months who were hospital patients with acute and chronic infections were studied similarly. While fed breast milk, their average gastric pH was 4.74. After a feeding of undiluted whole milk the pH was 5.35, slightly higher than in the normal infants discussed above. These authors reported that at pH 5 marked inhibition of the growth of members of the colon, dysentery and typhoid group of bacilli occurs. At pH 4 complete inhibition or death of these microorganisms takes place.

Bartholomew et al. (1980) studied microbial growth and nitrite formation in the stomachs of an unspecified number of patients with pernicious anemia and 31 ulcer patients being treated with a drug to reduce gastric acidity. Details of their assays were not given. They found a relation between the pH of the gastric contents and the numbers of bacteria present. Also a close relationship was found between the pH and the percentage of nitrate reduced to nitrite in the gastric contents. They concluded that when the pH is above 5 there is a relatively rich flora and a relatively high nitrate reductase activity.

3. Metabolism of Absorbed Nitrite

Burrows (1979) characterized the action of nitrite in blood on hemoglobin using mature ewes of mixed breeding. A 3% solution of sodium nitrite in sterile physiological saline was administered intravenously in doses of 6.6 to 100 mg/kg to groups of four ewes. The nitrite ion oxidized divalent ferrous hemoglobin to trivalent ferric methemoglobin, which is incapable of transporting molecular oxygen. The administration of various doses of sodium nitrite resulted in the gradual formation of up to 80% methemoglobin. Doses of 6.6, 22, 35, or 50 mg/kg resulted in maximum methemoglobin levels of 13% at 15 minutes, 43% at 45 minutes, 63% at 60 minutes, or 80% just before death after 60 minutes, respectively. A reductase enzyme system in the red blood cells slowly reduces methemoglobin back to functional hemoglobin, but this can be enhanced by administering the antidote methylene blue. Groups of four ewes were given intravenous methylene blue 30 minutes after 50 mg/kg sodium nitrite at doses of 2.2, 4.4 or 22 mg/kg. Methemoglobin levels dropped abruptly in a dose-dependent manner and all animals survived.

The normal methemoglobin level in human blood is variously reported between 0.5 and 2.5% of the total hemoglobin. However, Skrivan (1971) determined methemoglobin levels of 100 pregnant women at two-week intervals. There was a consistent elevation of methemoglobin concentration from the 14th week of pregnancy through delivery, with the average level rising from 2% at the twelfth week to 10.5% at the 30th week. This was followed by a decline to 5.5% at the 38th week. All levels returned to normal after delivery. Women around the 30th week of pregnancy would be unusually sensitive to nitrite induction of clinical methemoglobinemia.

Gruener et al. (1973) determined activity of methemoglobin reductase in six pregnant rats (strain not given), 10 rat fetuses, 49 pregnant women, and cord blood of 69 human newborns. The rat fetuses had activity nearly ten times higher than that of adult rats or that found in human cord blood. Human adult blood had 1.5 times the activity of the human cord blood. These findings indicate the possibility that the human fetus might have a weaker defense mechanism against intrauterine exposure to nitrites than that detected in rats. In rats nitrite has been shown to cross the placenta.

4. Reactions of Nitrite in the Stomach

Sander et al. (1968) demonstrated the reaction of nitrite with secondary amines to form nitrosamines in the stomachs of Sprague-Dawley rats. Twenty-four rats were fed a standard diet (amount not reported) with 0.01% diphenylamine and 0.15% sodium nitrite and killed after 30, 60, 120, or 180 minutes. Stomach contents were extracted with dichloromethane for two-dimensional chromatography to detect diphenylnitrosamine. The amount found reached a maximum of 31% of theoretical at 120 minutes, presented as 37 μg/g of stomach contents. *In vitro* studies indicated that the reaction is optimum at pH 1 to 3. This corresponds to the conditions found in the stomach of humans and various experimental animals. Weakly basic amines were converted into nitroso compounds up to a thousand times more rapidly than strongly basic amines, which could not be shown to nitrosate in the rat stomach.

Mirvish et al. (1973) determined the kinetics of nitrosation of the amino acids proline, hydroxyproline and sarcosine by sodium nitrite in vitro using perchloric acid to control the reaction pH. The authors found a pH optimum of 2.5. Thiocyanate increased the rate of sarcosine nitrosation 10- to 400-fold, especially at low pH.

Ohshima and Bartsch (1981) determined endogenous formation of N-nitrosoproline in a male volunteer who ingested 0 to 325 mg nitrate in red beet juice (0 to 4.64 mg/kg) followed 30 minutes later by 0 to 500 mg proline (0 to 7.14 mg/kg). Urine was monitored for N-nitroso compounds. The amount of nitrosoproline excreted was found to be proportional to the proline dose and increased exponentially with the nitrate dose ingested. Neither nitrate nor proline taken alone led to a detectable increase. The man's normal excretion of nitrosoproline was <3 μg/L. Administration of nitrate followed by proline increased this to a maximum of 17 to 30 μg/L at the highest doses tested. This was a good test system for demonstrating nitrosation in the human stomach because proline is an amino acid found in most protein foods, and nitrosoproline is not volatile nor carcinogenic in animals.

Fine et al. (1983) compared the in vivo data of Ohshima and Bartsch (1981) with the in vitro data of Mirvish (1975). The in vivo data fall within theoretical limits predicted if 0.5% to 1.5% of ingested nitrate is reduced to nitrite per hour in human saliva. This study indicates that the extent of in vivo formation of other more harmful N-nitroso compounds in the human stomach can be predicted from in vitro kinetic studies.

Archer et al. (1975) demonstrated the ability of ascorbic acid to block nitrosation of morpholine in vitro by rapidly reducing nitrite to nitric oxide or nitrogen. The reaction kinetics are dependent on the redox potential of the medium. Under anaerobic conditions, one mole of ascorbate will reduce two moles of nitrite. In the presence of bubbling air equimolar ascorbate is required. These experiments were conducted at pH 3, 3.5 and 4 maintained by perchloric acid.

Similar in vitro studies were done by Kamm et al. (1977) showing the ability of α-tocopherol (vitamin E) to remove nitrite ion from simulated gastric fluid. The rate of reaction was shown to be optimum at pH 2 to 3 and negligible at pH 5. The α-tocopherol reacted more readily than ascorbate to destroy nitrite ion at pH values below 4. Appropriate controls were used by these authors.

Ohshima and Bartsch (1981) confirmed the inhibitory value of ascorbic acid and α-tocopherol in the human volunteer protocol presented above. Intake of 1 gram of ascorbic acid simultaneously with 325 mg nitrate and 250 mg proline reduced the nitrosoproline concentration in urine to the control level. Five hundred mg of α-tocopherol inhibited nitrosation only about 50%.

D. Excretion

Keith et al. (1930) studied excretion of nitrates in four young men. After ingestion of 10 grams of ammonium nitrate, a measurable amount of nitrate could not be detected in the feces. Seventy-nine percent of the nitrate ingested was excreted promptly, and a total of 88% was recovered within three to four days. Nitrate was assayed with a colorimetric test using diphenylbenzidine.

Tannenbaum et al. (1980) recorded urinary excretion of nitrate by individuals on a low-nitrate soy diet for 84 days. The average daily excretion was about six times the nitrate intake. The data suggest a fairly constant introduction of extra-dietary nitrate into the system. Based on these data and the authors' previous demonstration of nitrite in ileostomy contents in the absence of nitrate, the authors conclude that the most likely explanation is that gut microorganisms oxidize reduced forms of nitrogen, such as ammonium compounds, to nitrite in the intestinal tract. This nitrite is absorbed and is then converted to nitrate by the process of methemoglobin formation and reduction (*de novo* synthesis).

Green et al. (1981) studied nitrate excretion in germ-free or conventional male Sprague-Dawley rats weighing 150 grams. The basal diet was supplemented with 0%, 0.001% or 0.005% $Na^{15}NO_3$ (0, 0.5 or 2.5 mg/kg/day). Urine and feces were collected during 24-hour intervals. Nitrate content was determined by reducing it to nitrite and assaying by diazotization and coupling with a

Griess reagent. Isotope levels were determined by conversion to nitrobenzene and analysis by gas chromatography-mass spectrometry. Rats not supplemented with sodium nitrate excreted ten times more nitrate in the urine than they ingested. There was no difference between germ-free and conventional rats. This indicates that endogenous nitrate synthesis is not due to intestinal flora. Rats supplemented with nitrate at the lower dose (0.5 mg/kg/day) excreted slightly more nitrate than they ingested. The rats supplemented at the higher dose (2.5 mg/kg/day) excreted slightly less nitrate than they ingested. The possibility of a body pool of nitrate turning over during the experiment was ruled out. After stopping supplementation no $^{15}NO_3$ was detectable in the urine on the third day. The authors could not detect nitrate in feces of conventional rats.

Schneider and Yeary (1975) studied the kinetics of the elimination of nitrite from the plasma of seven dogs of both sexes and mixed breeds, seven cross-bred ewes, and seven Shetland ponies of both sexes. Sodium nitrite was administered intravenously at 20 mg/kg and blood was collected 15 to 30 seconds after injection, at 1-minute intervals between 1 and 10 minutes, at 5-minute intervals between 10 and 30 minutes, at 10-minute intervals between 30 and 60 minutes, and then at 1.5, 2, 3, 6 and 24 hours after injection. A colorimetric assay for nitrite was used after plasma was isolated. The half-life for elimination of nitrite from plasma was between 0.5 and 0.6 hours in all three species. The authors concluded that this rapid clearance indicated a process other than renal excretion, such as metabolic conversion to nitrate.

Donahoe (1949) reported five cases of infant methemoglobinemia in infants less than three-months-old. These included one case in a newborn breast-fed infant and one case in a young infant fed milk from cows drinking nitrate-contaminated water. This indicates excretion of nitrate into milk of humans and cows.

Davison et al. (1964) added nitrate to the diets of Holstein heifers during pregnancy. Milk samples were taken at weekly intervals from parturition to 30 days. Milk from heifers fed normal rations averaged 5 ppm nitrate, while milk from heifers fed 440 mg/kg/day and 660 mg/kg/day nitrate averaged 9 ppm and 15 ppm nitrate, respectively. In contrast, Crowley et al. (1974) reported a long-term study of a Wisconsin dairy herd drinking water with 374 ppm nitrate. The Holstein cows produced milk containing an average of 5.7 ppm nitrate. This compared to controls drinking water containing 19 ppm nitrate which produced milk containing 5.6 ppm nitrate.

E. Bioaccumulation and Retention

No evidence was found for bioaccumulation of nitrate or nitrite in any tissue storage depot. However, nitrate is retained by recycling through saliva with reabsorption into plasma as explained in III.B. (Spiegelhalder et al. 1976).

F. Summary

The primary route of nitrate and nitrite absorption is through the gastrointestinal tract. Nitrate is absorbed by active transport through the wall of the upper small intestine. Nitrite is absorbed by diffusion across the gastric mucosa and also through the wall of the intestinal tract. In both cases, absorption following ingestion is essentially complete (i.e., 100%). Nitrate and nitrite can also be absorbed into the blood through the lungs or can be absorbed following formation in the lungs after inhalation of nitrogen oxides.

Nitrate is rapidly disseminated throughout the body in the blood and is actively secreted in saliva, gastric juices, and urine. Nitrite reacts so rapidly with hemoglobin that little is transported to the tissues. Nitrate and nitrite do not bioaccumulate. However, they are constantly present in the body as a result of de novo synthesis as well as ingestion.

Nitrate is reduced to nitrite by the action of oral bacteria on salivary nitrates. Approximately 20% to 25% of ingested nitrate is secreted in salvia and approximately 20% of the secreted nitrate is transformed to nitrite by oral bacteria in healthy human adults and older children. The same process occurs in the stomach of young infants and others with abnormally high pH in the stomach. In the infant, approximately 100% of ingested nitrate is transformed to nitrite by this process. Nitrite reacts with hemoglobin, oxidizing it to methemoglobin. During enzymatic repair of this injury, the nitrite is oxidized to nitrate. Nitrite in the stomach reacts with secondary amines and other amino substrates to form N-nitroso compounds which are readily absorbed.

Nitrate is excreted readily through the kidneys and can also be excreted in the milk of cows and women. When excess nitrate is not added to the diet, rats excrete significantly more nitrate than they ingest. Nitrite is so reactive that none remains to be excreted.

Figure III-1 summarizes the absorption, distribution, sites of metabolism, and excretion of nitrate and nitrite ions.

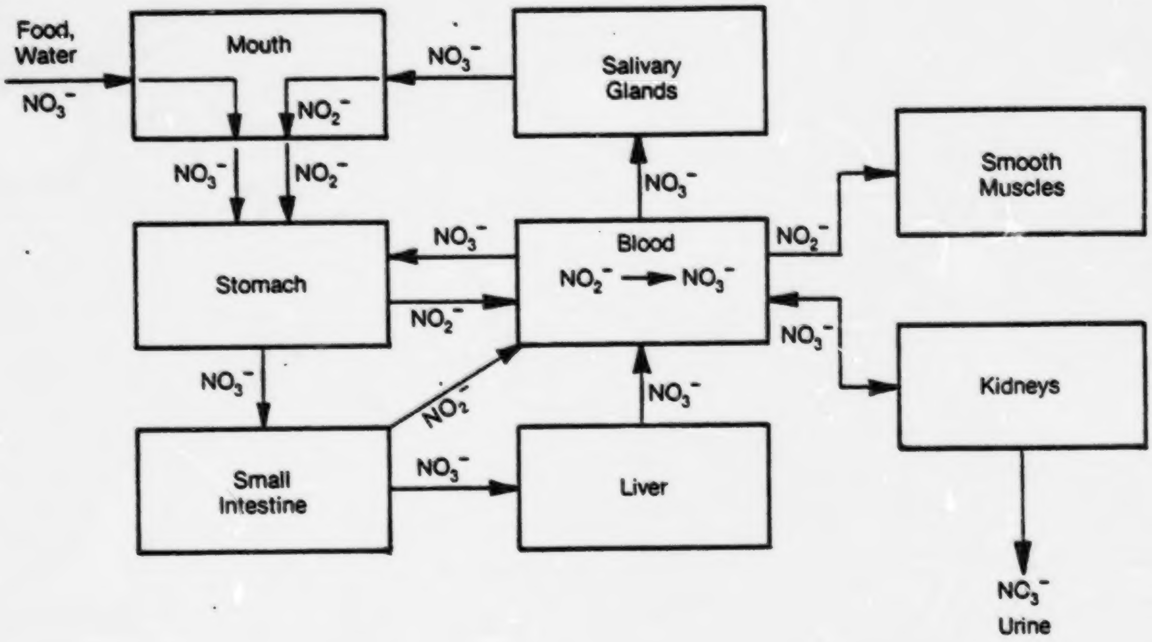
Food,
Water
NO_3^-
Mouth
NO_2^-
NO_3^-
Salivary Glands
NO_3^-
NO_2^-
NO_3^-
Stomach
NO_3^-
Blood
$NO_2^- \rightarrow NO_3^-$
NO_2^-
Smooth Muscles
NO_2^-
NO_3^-
Kidneys
NO_3^-
NO_2^-
NO_3^-
Small Intestine
NO_3^-
Liver
NO_3^-
Urine

Figure III-1 Metabolism of Nitrate and Nitrite

IV. HUMAN EXPOSURE

Humans may be exposed to nitrate (expressed as nitrate-N) in drinking water, food, and air. Detailed information concerning the occurrence of and exposure to nitrate in the environment is presented in another document entitled "Occurrence of Nitrate in Drinking Water, Food, and Air" (Taylor et al. 1985). This section summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food, and air.

Exposure Estimation

This analysis is limited to drinking water, food, and air, since these media are considered to be general sources common to all individuals. Some individuals may be exposed to nitrate from sources other than the three considered here. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and several factors over which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Unfortunately, data and methods to estimate exposure of identifiable population subgroups from all sources simultaneously have not yet been developed. To the extent possible, estimates are provided of the number of individuals exposed to each medium at various nitrate concentrations. The 70-kg man is used for estimating intake.

a. Drinking Water

There is very limited data available for characterizing human intake of nitrate (expressed as nitrate-N) from drinking water provided by public water supplies. Based on the data presented in Taylor et al. (1985), it appears that most of the approximately 214 million people in the United States using public water supplies are exposed to levels below 5 mg/l nitrate-N. While some may be exposed to drinking water with no nitrate present, information available from one survey suggests that most supplies do have nitrate present mg/l. Consequently, a detailed relative source contribution assessment that describes the importance of each medium as a nitrate source for different segments of the U.S. population could not be developed.

While it is not possible to develop a detailed profile of human exposure and the relative source contribution, it is possible to describe the relative importance of the three media for typical exposure conditions.

The available information strongly indicates that food is the predominant source of nitrate (expressed as nitrate-N) for most individuals. Using food values based on estimates developed from dietary consumption data for the average U.S. population by the National Academy of Science's Committee of Nitrite and Alternative Curing Agents in Food, the dietary intake of nitrate for the adult male is estimated to range from 16.9 mg/day nitrate-N for intake from all exogenous dietary food sources, to 60 mg/day nitrate-N for intake from a vegetarian diet. Assuming 100% absorption and a body weight of 70 kg, the nitrate-N dose from food ranges between 0.24 and 0.85 mg/kg/day. Compared to the dose from food, the dose from air is relatively insignificant: assuming a daily intake of 1.5 mg (see Section 5) and an absorption rate of 100%, the respiratory nitrate-N dose is only 0.005 mg/kg/day, which is less than 2% of the dose from food when all exogenous sources are considered and less than 0.5% when only vegetarian sources are considered. Drinking water becomes the predominant source of nitrate-N at levels above 10 mg/l, when all food sources are considered. For vegetarians, drinking water becomes the predominant source of nitrate at levels at or above 50 mg/l.

at levels above 0.01 mg/l nitrate-N. There are too few data to reliably estimate the number of persons exposed to nitrate in drinking water at various levels below 10 mg/l, the current MCL. However, based on the compliance monitoring data available through FRDS, it can be estimated that approximately 2 million people are receiving drinking water from public water supplies with nitrate levels above 10 mg/l, of which approximately 66% consume water from groundwater supplies.

b. Diet

Several estimates have been made of daily intake of nitrate (expressed as nitrate-N) in the United States. These are presented in Table IV-1. The estimates generally place daily intake in the range of 8.8-22.6 mg nitrate-N for adults. The largest source of nitrate was estimated to be from vegetables, accounting for 86-95% of the daily nitrate intake. Cured meats provide an additional 10% of nitrate intake. Cured meats also provide between 61.5% and 92% of daily dietary nitrite intake. Adult dietary intake of nitrite (expressed as nitrite-N) is estimated to range from 0.1-0.8 mg/day.

c. Air

The Committee on Nitrite and Alternative Curing Agents in Foods (NAS 1981) estimated an average daily respiratory intake of nitrate (expressed as nitrate-N) based on an average nitrogen dioxide concentration of 58 ug/m^3 and assuming that 100% of the nitrogen dioxide is converted to nitrate. The estimated daily average adult intake would be 1.5 mg nitrate-N. Relative to the intake of nitrate from food and drinking water, the intake from air appears to be insignificant.

Summary

There is a limited data base on human exposure to nitrate from drinking water, food, and air. While some information is available to suggest the general range of nitrate intake from these three media, it is not possible to determine what proportion of the population have drinking water, dietary, and respiratory intakes at various levels. For drinking water, it is possible to estimate the size of the population exposed to levels in public water supplies above 10 mg/l, the current MCL, but not for various levels at or below 10

Table IV-1. Estimate of Per Capita Daily Dietary Nitrate[a] Intake by U.S. Residents

	Intake, mg/person/day (percentage of total daily intake from all sources), by study			
Food	White 1975, 1976	FDA 1979	Hartman 1981	Birdsall 1981
Cured meats	2.1 (9.4)	0[b] (0)	0.01 (0.6)	2.1 (9.4)
Vegetables	19.4 (86)	8.4 (95)	13.0 (91.7)	19.4 (86)
Fruits and juices	0.3 (1.4)	NR[c]	0.3 (1.8)	0.3 (1.4)
Bread	0.5 (20)	NR	0.5 (2.8)	0.5 (2.0)
Milk and milk products	0.05 (0.2)	NR	0.05 (0.3)	0.05 (0.2)
Water	0.2 (0.7)	0.5 (5)	0.5 (2.8)	0.2 (0.7)
Total	22.6 (100)	8.8 (100)	15.8 (100)	22.6 (100)

[a] Expressed as NO_3-N = mg NO_3^-/4.43.

[b] 0 indicates a dietary contribution that is relatively unimportant in comparison with other food categories listed.

[c] NR = no data reported.

Source: NAS 1981

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Taylor K, Borum D, Letkiewicz F. 1985. Occurrence of nitrate in drinking water, food, and air. Prepared by SAIC/JRB Associates, McLean, VA, for Office of Drinking Water, U.S. Environmental Protection Agency.

V. HEALTH EFFECTS IN ANIMALS

A. Acute Toxicity

Lethal dose data reported for nitrate and nitrite in various species are summarized in Table V-1. The toxic effects of nitrate are due to its reduction to nitrite. Ruminants are especially susceptible to nitrate poisoning because bacteria in the rumen reduce the ingested nitrate to nitrite (Emerick 1974). The toxicity of the nitrite formed in the digestive tract is affected by the amount and composition of the stomach contents. This is because reaction with the stomach contents can reduce the dose absorbed. For this reason, ruminants receiving grain or other sources of readily available carbohydrates have been shown to be more tolerant to nitrate than those on low quality forage (Emerick 1974).

Tolerance to nitrite has been induced experimentally in lambs by Sokolowski et al. (1960). Six lambs averaging 32 kg received 0, 932, or 1,243 mg/kg nitrate (as the potassium salt) in their rations for 129 days. Subsequently, levels of nitrate up to twice the highest dose were fed without causing any visible symptoms of nitrite toxicity.

In nonruminant animals, nitrate is toxic only if it is excreted in saliva and reduced to nitrite by oral bacteria. The nitrite which does not react with stomach contents is then absorbed. Dogs appear to be unable to reduce nitrate. This suggests that they do not secrete nitrate into saliva. No

Table V-1 Toxic Doses of Nitrate and Nitrite - Oral

Animal	Effect	Cation	Dose of NO_3^- (mg/kg)	Dose of NO_2^- (mg/kg)	Reference
Rat	LD_{50}	Na^+		100	a
Rat	LD_{50}	Na^+		120	b
Rat	LD_{50}	K^+	1,986		c
Rat	LD_{50}	K^+		46	c
Rat (1-yr)					
fasting	LD_{50}	Na^+		51	d
with food	LD_{50}	Na^+		87	d
Rat (3-mo)					
fasting	LD_{50}	Na^+		73	d
Mouse	LD_{50}	K^+		119	c
Mouse	LD_{50}	K^+		95	c
Rabbit	LD_{50}	K^+	1,166	108	b
Rabbit	LD	Na^+		53-60	e
Rabbit	LD_{50}	Na^+	1,955		b
Pig	LD	Na^+		60	e
Pig	LD	Na^+		59	e
Cow	LD	K^+	614		e
Cow	LD	Na^+	328		e
Cow	LD	Na^+	332		e
Cow	LD	Na^+	620		e
Sheep	LD	Na^+	310	111	e

[a] Imaizumi et al. 1980
[b] Merck Index 1976
[c] WHO 1962
[d] Druckrey et al. 1963
[e] Burden 1961

toxic effects were produced in female dogs given 3,485 mg/kg intravenously or 1,700 mg/kg orally in one day (Greene and Hiatt 1954).

Poisoning of pigs by nitrate administered in a drench was accomplished only at levels exceeding 300 mg nitrate-nitrogen (1,350 mg NO_3^-/kg body weight). Death was attributed to erosion and hemorrhage of the gastric mucosa rather than from nitrite toxicity (Emerick 1974).

Sheep showed no toxicity after intravenous administration of 2,500 mg/kg potassium nitrate (Pfander et al. 1957). This result indicates that none of the nitrate entered the rumen where it could be reduced to nitrite and suggests the absence of nitrate in the saliva of sheep.

The acute toxicity of absorbed nitrite takes two forms which vary greatly in importance among species, as the smooth muscles and red blood cells compete for the nitrite ion.

1. Cardiovascular Effect

The nitrite ion is a relaxant for smooth muscles and dilates blood vessels. Vasodilator effects are predominant in species whose hemoglobin reacts with nitrite slowly. Most of the nitrite remains circulating to the target smooth muscles. Bartik (1964) studied this effect in the horse. After intravenous administration of 3.7 mg/kg sodium nitrite, rapid pulse from low blood pressure peaked in five minutes. The methemoglobin level peaked 30 minutes after administration. Clinical symptoms of methemoglobinemia cannot be studied in the horse unless the lethal vasodilator effect is first mitigated by administration of epinephrine (Bartik 1964).

2. Effect on Hemoglobin

Nitrite ion circulating in the blood oxidizes hemoglobin in the red blood cells. This increases the level of methemoglobin, which cannot reversibly bind oxygen. Methemoglobinemia is characterized clinically by cyanosis and anoxia. In sheep, death generally occurs after 70% to 80% of the hemoglobin is converted (Burrows 1979). Details of this study were presented in Section III.C. of this document.

Smith and Beutler (1966) studied species differences in the rate of methemoglobin formation and its reduction to oxyhemoglobin. The authors used an *in vitro* assay with sodium nitrite as the oxidant and glucose as the substrate for reduction. Both the rate of oxidation of hemoglobin and the rate of reduction of methemoglobin showed wide species differences, but the two rates were roughly proportional. Sheep and goats had the fastest rates for both processes followed by humans, horses, and pigs in order of decreasing rate. In cows, the rate for methemoglobin formation was faster than in humans. The reduction rate in cows was slower than in humans. Therefore, cows are more susceptible than humans to the symptoms of methemoglobinemia (Smith and Beutler 1966). After oral administration of nitrate to the horse, maximum concentration of methemoglobin appeared 20 hours later (Bartik 1964).

In the rat the peak level appeared 30 minutes after an oral dose (Shuval and Gruener 1977). Rats are quite resistant to lethal methemoglobinemia induced by nitrate or nitrite ingestion (see Table V-1). After administration of the LD_{50} dose to rats, the methemoglobin level rose to 45% to 80% in one hour and, in the survivors, returned to normal after 24 hours (Imaizumi et al. 1980).

3. Toxicity with Nitrosatable Substrates

Liver necrosis was observed when 1,000 mg/kg dimethylamine and 100 mg/kg sodium nitrite were given simultaneously to adult male Charles River mice by gavage. The necrosis was attributed to intragastric formation of nitrosodimethylamine (Asahina et al. 1971). Liver injury was reduced when the nitrite was given 15 to 120 minutes before, but not three hours after, the dimethylamine. This suggests that nitrite disappeared fairly rapidly from the stomach. Similarly, gavage of four male MRC rats weighing 200 grams with 175 mg/kg of aminopyrine and 50 mg/kg of sodium nitrite three times daily produced liver necrosis. Presumably this was due to the formation of nitrosodimethylamine (Lijinsky and Greenblatt 1972). The liver necrosis was detected histologically and by the rise in serum glutamic pyruvic transaminase (SGPT).

Acute nitrosamine toxicity in the liver has been prevented by simultaneous administration of ascorbic acid with the nitrite and amine (Greenblatt 1973, Kamm et al. 1975). Ascorbate prevents toxicity only when it is present in the stomach of the rat with the amine and nitrite. Erythorbic acid (Kamm et al. 1975) and an emulsion of a-tocopherol (vitamin E) (Kamm et al. 1977) are as effective as ascorbic acid in preventing hepatic necrosis. Complete protection from measurable liver toxicity (elevation of SGPT) required that the inhibitor be at least equimolar with the nitrite (Kamm et al. 1977). Prevention of histologically observed necrosis required twice the amount of ascorbic acid (Greenblatt 1973). The vitamins are oxidized in the process of inactivating nitrite. The common antioxidants BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) had no measurable protective value at 0.6 equimolar (Astill and Mulligan 1977).

B. Chronic Toxicity

1. Effect on Fluid Balance

Greene and Hiatt (1954) performed 30 experiments on 13 female dogs (strain and weight not given) to evaluate the effect of nitrate salts on electrolyte balance. Animals maintained on a low salt diet and administered repeated injections of nitrate showed evidence of dehydration. The signs consisted of dry eyes, dry mucous membranes, rapid pulse, anorexia and listlessness. One 14-kg dog was administered a daily dose of 930 mg/kg sodium nitrate intravenously for eight days. A 36% decrease in plasma chloride, 14% decrease in plasma sodium and a 1.2 kg loss of weight were observed.

Ten male Sprague-Dawley rats initially weighing 50 grams were fed 2,500 mg/kg/day sodium nitrate in the diet for six months. The results showed a statistically significant ($P<0.01$) diuretic effect by two months when compared to rats fed equimolar sodium chloride. Rats fed one-tenth of that dose showed no significant diuresis (Fritsch et al. 1980).

2. Effect on Vitamin Absorption

O'Dell et al. (1960) studied the effect of nitrite on vitamin absorption in rats of unreported strain. Two groups of 12 weanling rats were used. Group I received a vitamin A-deficient basal diet and group II received the basal diet plus 0.3% potassium nitrite (150 mg/kg/day). A period of six to eight weeks was required for depletion of stored Vitamin A in animals fed either diet. At this time xerophthalmia was evident in Group II and growth in both groups had stopped. Rats in Group I had grown more rapidly than those in Group II. Liver vitamin A analysis correlated with the growth data of the two rats sacrificed in each group. Each group was then divided into subgroups (IA, IB and IIA, IIB). Subgroups IA and IIA received the basal diet plus beta-carotene at 1 mg/100 g diet (0.5 mg/kg/day). Subgroups IB and IIB received the basal diet plus beta-carotene and 0.3% potassium nitrite. As in the first study period, growth was depressed by potassium nitrite. After four weeks with carotene added (ten weeks from start of depletion) rats of Subgroups IIA and IIB showed a dystrophy typical of vitamin E deficiency. Not only did nitrite cause a more rapid depletion of vitamin A but it precipitated a vitamin E deficiency in the animals given a diet normally adequate in vitamin E (5 mg/100 g diet, or 2.5 mg/kg/day). Further evidence for the vitamin E deficiency was shown by the improvement of appetite and muscular coordination when vitamin E concentrate was given to the rats in Subgroups IIA and IIB. In an *in vitro* experiment, Olson et al. (1963) demonstrated that nitrite in an acid medium caused rapid beta-carotene disappearance. Pugh and Garner (1963) confirmed this and also reported destruction of vitamin A alcohol.

The destruction of vitamin A and carotene by nitrite in the stomach has not been observed in ruminants. Presumably this is because nitrite is absorbed from the rumen where the pH is too high for the reaction of nitrite with the vitamin and its precursor. Davison et al. (1964) fed dairy heifers nitrate at levels of 440 or 660 mg/kg/day (15 at each dose and 15 controls) for five months or more and found no difference in vitamin A or carotene nutrition.

Chow et al. (1980) studied groups of 10 and 12 two-month-old male Sprague-Dawley rats provided drinking water containing 4,000 ppm sodium nitrate (267 mg/kg/day) or 2,000 ppm sodium nitrite (133 mg/kg/day). A significant ($P<0.05$) decrease in plasma vitamin E level compared to controls (nine rats) was found after 14 months. Nitrate and nitrite lowered the plasma vitamin E content by 29% and 57% respectively. A similar experiment using one-tenth of the above dose for four months showed no significant change (P value not given).

3. Effect on Internal Organ Morphology

Shuval and Gruener (1972) reported a study of three-month-old male rats (strain not given) in groups of eight given sodium nitrite in drinking water at 0, 100, 1,000, 2,000 or 3,000 mg/L (approximately 0, 13, 133, 267 or 400 mg/kg/day) for 24 months. This was followed by histologic examination of tissues from the heart, lungs, kidneys, liver, spleen, pancreas, adrenals and brains. The last three organs showed no pathological features in any of the rats. The liver and spleen were frequently congested while the kidneys sometimes showed focal inflammatory and degenerative changes. In the lungs, the bronchi were frequently dilated. The bronchial walls were infiltrated with lymphocytes and the mucosa and muscle were often atrophied. Purulent bronchial exudate also occurred. Interstitial round cells, fibrosis, and emphysema were sometimes encountered. Most of these effects were present in one or two rats of the control and low-dose groups but were found with increasing frequency and severity in the three groups treated with the highest doses. In the heart, degenerative foci were frequent in the highest-dose groups only. The intramural coronary arteries in all of the experimental groups, and especially in the highest-dose group, were thin and dilated. The controls showed some degree of thickening consistent with aging, with some even having a marked hypertrophy and narrowing.

The above experiment was repeated by Shuval and Gruener (1977) using 52 male albino Sabra rats per group. The sodium nitrite doses were similar except that the low dose was 200 mg/L (about 26 mg/kg/day). An additional 52 animals were treated with sodium nitrate at 2,000 mg/L (about 260 mg/kg/day). Histologic examinations were carried out on heart and lung taken from animals sacrificed at 3-month intervals. The nitrate group and the lowest dose nitrite group showed no increased methemoglobin levels. The highest methemoglobin levels seen in the 1,000, 2,000, and 3,000 mg/L nitrite groups were 4.44% at one month, 11.43% at one month, and 23.9% at two months respectively. Thinning and dilation of the coronary arteries was observed in approximately half of the rats administered the lowest sodium nitrite dose and most of the rats administered either nitrate or nitrite at the higher dose levels. The results of the histologic examinations on lung tissues were not reported by the authors.

Two long-term studies using ICR mice report significant increases in amyloidosis after ingestion of high doses of sodium nitrate or sodium nitrite. Sugiyama et al. (1979) fed groups of 50 male and 50 female mice sodium nitrate at 0, 2,500 or 5,000 mg/kg/day for more than a year. Amyloidosis was present in 37% of survivors at the high dose, in 42% at the lower dose, and in 25% of the controls. Inai et al. (1979) used groups of 50 male and 50 female mice given 0, 1,250, 2,500 or 5,000 mg sodium nitrite/L in drinking water (about 208, 416 or 833 mg/kg/day) for more than 18 months. The author reported that the liver was more affected by sodium nitrate than other organs and showed marked atrophy and hemosiderosis. Amyloidosis, however, was noted in various organs including the liver, spleen, kidneys, and adrenals. Quantitative data on these lesions were not reported.

Fritsch et al. (1980) administered sodium nitrate at 250 or 2,500 mg/kg/day or sodium nitrite at 25 or 250 mg/kg/day to groups of ten male Sprague-Dawley rats in a standard diet for six months. They observed changes in the spleen in all the treated animals. The changes included a significant release of ferric iron and the presence of hemorrhagic areas. The authors interpreted this as due to the chronic struggle of treated animals against methemoglobinemia.

4. Effect on Muscles

Shuval and Gruener (1977) treated groups of fifteen 50-day-old male C_{57}bl/6J mice with 0, 100, 1,000, 1,500 or 2,000 mg sodium nitrite/L of drinking water (reported to be 0, 8.8, 88, 133 or 178 mg/kg/day, respectively) for three weeks. Methemoglobin levels and motor activity were studied. No change was observed at 8.8 mg/kg/day and the changes at 88 mg/kg/day were insignificant. The dose of 133 mg/kg/day produce a significant increase in methemoglobin levels (above 2%, which was the P=0.05 level) and 178 mg/kg/day produced both a significant increase in methemoglobin levels and a significant decrease in motor activity. A possible explanation for this finding is that sodium nitrite can cause the conversion of myoglobin into metmyoglobin. This could interfere with muscle activity by lowering the oxygen capacity of the muscle tissues.

5. Effect on Central Nervous System

Groups of four male rats of the Sabra strain were treated with 0, 100, 300 or 2,000 mg sodium nitrite/L of drinking water for two months (Shuval and

Gruener 1977). Brain electrical activity was then studied during and following treatment. The high-dose rats consumed 280 mg/kg/day of sodium nitrite and showed altered brain electrical activity from the fourth day of treatment. Spikes and sharp waves appeared and the background activity increased to a maximum after two weeks. At this time general paroxysmal outbursts of high amplitude sharp waves became apparent. This pattern continued during the two month treatment period and during the 4.5-month post-exposure observation period. During treatment, these high-dose rats averaged 12% methemoglobin. This dropped quickly to less than 1% during the observation period. Rats in the high-dose group were sedate when compared with the control group as well as with their own behavior before the treatment. During the electrical outbursts their behavior suddenly changed and the rats remained motionless but awake. The middle-dose rats consumed 42 mg/kg/day and showed spikes and sharp waves after four days and paroxysmal outbursts after two weeks. These outbursts were less frequent but of the same amplitude as in the high-dose group. Outbursts continued to appear during a 3.5-month post-exposure observation period. The low-dose group consumed 14 mg/kg/day and showed changes similar to the other groups but less frequently. During the 2.5-month post-exposure observation period, outbursts stopped after two weeks but spikes and sharp waves continued.

A repeat of the above experiment using different staff, facilities and equipment but the same strain of rats detected no abnormalities of electrical brain-wave activity (Shuval and Gruener 1977). The authors were unable to explain this discrepancy.

C. Teratogenicity and Reproductive Effects

The U.S. Food and Drug Administration supported teratogenicity studies on sodium and potassium nitrates and nitrites in CD-1 mice, Wistar rats, golden hamsters, and Dutch belted rabbits (FDA 1972a, b, c, d). The doses and schedules for these studies are shown in Table V-2. The doses were administered by oral intubation to 20 to 26 pregnant rodents or 10 to 13 rabbits. Controls were sham treated; however, the solvent was not reported. Fetuses were delivered by Caesarean section on the scheduled day. One-third of the fetuses of each litter of mice, rats, or hamsters underwent detailed visceral examinations employing 10x magnification. The remaining two-thirds of the fetuses were cleared in potassium hydroxide, stained with alizarin red S dye and examined for skeletal defects. The live fetuses of each litter of rabbits were placed in an incubator for 24 hours for evaluation of neonatal survival. The survivors were then sacrificed, examined for visceral abnormalities by dissection, and then cleared and stained for examination for skeletal defects. No histopathology was done on any tissue of the fetuses or dams.

Data from all groups given nitrates and from mice and rabbits given nitrites were summarized as follows:

> "The administration of up to (highest dose tested) mg/kg of the test material to pregnant (species) for (number) consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls" (FDA 1972a pp. 1, 14, 27, 40; FDA 1972b pp. 1, 40; FDA 1972c pp. 1, 14, 27, 40; FDA 1972d pp. 1, 42).

Table V-2 FDA Teratogenicity Study Protocols

Compound	Dose in mg/kg/day			
	Mouse	Rat	Hamster	Rabbit
Sodium Nitrite	0.2	0.1	0.2	0.2
	1.1	0.5	1.1	1.1
	5.0	3.0	5.0	5.0
	23.0	10.0	23.0	23.0
Potassium Nitrite	0.3	0.1	0.3	0.2
	1.5	0.5	1.5	1.1
	7.0	3.0	7.0	5.0
	32.0	10.0	32.0	23.0
Sodium Nitrate	4	2.5	4	2.5
	20	12	20	12
	100	54	100	91
	400	250	400	250
Potassium Nitrate	4	2	3	2
	20	9	20	10
	100	40	70	50
	400	180	280	206
Days treated	6-15	6-15	6-10	6-18
Day sacrificed	17	20	14	29

Adapted from FDA 1972 a,b,c,d

Reports on tests of nitrites in rats contained the above statement in regard to the three lowest doses only. The highest dose in rats was reported as follows:

> "At a dose level of 10.0 mg/kg, there were slight indications of delayed skeletal maturation, particularly with respect to ribs and skull. Outright increase in terata were not observed nor did this dose cause any alteration in nidation or fetal survival. It was concluded that this test material is not a frank teratogen for the rat under the experimental conditions employed" (FDA 1972b,d p. 14).

Reports on tests of nitrites in hamsters contained the standard statement followed by:

> "The administration of this test material appeared to be associated with delayed skeletal maturation in this species which was not clearly dose-related. Whether or not the substance is teratogenic in the hamster can only be determined on the basis of further studies" (FDA 1972b p. 26, FDA 1972d p. 28).

No information was given in the FDA reports as to the temporal relationship between feeding and dosing. Also the diet used was not defined, specifically whether it contained fish meal, which is a source of nitrosatable amines. The study gives no information on whether the observed immature skeletal development would be repaired during the neonatal period, or whether there was any relation between fetal immaturity and maternal methemoglobinemia. Statistical analysis of the data is not mentioned in the reports.

Sinha and Sleight (1971) reported studies in which four pregnant guinea pigs were administered sodium nitrite at 50 mg/kg subcutaneously on the 47th day of pregnancy. These animals underwent normal parturition. However, fetal deaths followed by abortion occurred in three guinea pigs administered sodium nitrite in the same manner at 60 mg/kg. The fetal deaths occurred approximately one hour after nitrite administration. This is when the maternal and fetal methemoglobin levels were highest. This result was prevented in similarly treated animals by injection of 10 mg/kg of methylene blue simultaneously with nitrite. The data suggest that fetal death resulted from hypoxia induced by maternal methemoglobinemia. Four other 47-day pregnant guinea pigs administered 70 mg/kg sodium nitrite similarly died within an hour.

Globus and Samuel (1978) studied the effect of oral sodium nitrite during pregnancy on the fetal hematopoietic tissues in CD-1 mice. Groups of 36 treated and 39 control mice were intubated with 0 or 16.7 mg/kg/day from day 0 until sacrifice. The treated and control mice were divided into subgroups that were sacrificed on the 14th, 16th or 18th day of gestation. Analysis of fetal livers indicated that maternally administered sodium nitrite stimulates fetal hepatic erythropoiesis. After removal of viscera, the fetuses were fixed and stained for skeletal examination. Fetal mortality, fetal resorption, the mean number of offspring per litter, the mean weight per fetus, and the incidence of skeletal malformations were not significantly different from controls (P value not stated).

Numerous studies of livestock have failed to show any relation between nitrate ingestion and abortion or other reproductive difficulties at doses below those causing severe maternal methemoglobinemia. The studies were done to clarify earlier field observations which had suggested this possibility (Emerick 1974). Farm animals studied in controlled experiments included cows, sheep, and swine. Parameters studied included conception rate, growth, length of estrous cycles, length of gestation, birth weight and performance of young, milk production, hemoglobin, retained placentas, and cystic ovaries. Davison et al. (1964) fed Holstein heifers nitrate at levels of 440 or 660 mg/kg/day (15 at each dose and 15 controls). Treated animals were divided into groups of five which started treatment at three times: before conception, 40 days pregnant, and 150 days pregnant. The conception rate was lower in high-dose heifers. In addition, two high-dose and one lower-dose heifers aborted and two high-dose heifers died. Other reproductive parameters listed above were not affected.

Experiments with Friesian cows during parturition showed that 12 mg/kg nitrite administered intravenously or 30 mg/kg administered orally to the dam caused much higher methemoglobin percentages and nitrite contents in the maternal blood than in the fetal blood. The ratio of nitrite content to methemoglobin content was similar in dam and fetal blood (Malestein et al. 1980).

Shuval and Gruener (1977) studied the transfer of nitrite to the fetus and the production of fetal methemoglobinemia in rats (strain "sabra"). The results are shown in Table V-3. After oral administration of a 30 mg/kg dose of sodium nitrite to the pregnant rat (day of pregnancy not reported), nitrite levels rose in the fetal blood with a lag of about 20 minutes behind the dam.

Table V-3 Relation of Blood Nitrite to Methemoglobin in the Rat

NaNO$_2$ dose mg/kg	Maximum metHb levels as percent of total Hb		Maximum nitrite levels in blood as NaNO$_2$ ug/mL	
	Mother	Fetus	Mother	Fetus
0.0	0.9	1.2	0.0	0.0
2.5	3.4	1.9	3.9	traces
5.0	5.0	2.7	6.9	traces
10.0	11.9	5.1	8.9	0.4
15.0	17.0	7.9	10.8	1.2
20.0	33.2	13.3	21.7	5.9
25.0	40.4	19.2	25.6	6.9
30.0	60.2	27.2	32.5	9.4

Adapted from Shuval and Gruener 1977.

This rise in nitrite in the fetus was followed by a rise in methemoglobin. All pregnant rats given doses of 60 mg/kg sodium nitrite died within one hour. Non-pregnant females survived such doses, indicating that pregnant rats have a higher susceptibility to nitrite toxicity.

Shuval and Gruener (1972, 1977) studied the effects of sodium nitrite in the drinking water on pregnant rats and their newborns. Groups of 12 pregnant rats (strain "sabra") were given water containing 2,000 or 3,000 mg/L (about 267 or 400 mg/kg/day) sodium nitrite through the third week after parturition. Seven controls drank tap water. Mean methemoglobin values were 24%, 5.5% and 1.1% for the high-dose, low-dose, and control groups, respectively. There was a pronounced effect on mortality among newborn rats of treated dams. Birth weights were similar in all groups, but mortality during the first three weeks of neonatal life was 53%, 30%, and 6% in the high-dose, low-dose and control groups respectively. After birth, pups of treated dams lagged in growth rate. At the end of 21 days, mean weight of the control group was 51.5 g compared to 29.5 g and 18.5 g in the low- and high-dose treated groups, respectively. The fur of pups in the treated groups thinned and lost its luster. From birth to weaning, pups in the treated groups showed no abnormally high methemoglobin, but total hemoglobin was 20% lower than controls.

Shuval and Gruener (1977) studied levels of methemoglobin reductase in the red blood cells of pregnant rats, fetal rats, pregnant women and human cord blood. The results are presented in Table V-4. Enzyme activity was

Table V-4 Methemoglobin Reductase Activity in Rat Fetuses and Pregnant Females - Rats and Humans

	Number	Methemoglobin reductase mμmoles/min/mg Hb
Pregnant rats	6	1.86±0.34
Rat fetus	10	17.4±1.3
Human pregnant females	49	2.38±0.78
Human cord blood	69	1.58±0.54

Adapted from Shuval and Gruener (1977).

assayed in a 10 μL blood sample which was incubated in a mixture containing citrate buffer, EDTA, $K_3Fe(CN)_6$ and MetHb. The reaction was started by adding NADH and was followed at 577 nm. The study showed that MetHb reductase activity is ten times higher in rat fetuses than in pregnant rats or human cord blood, while blood of pregnant women exhibits one and one-half times the activity of the cord blood.

A three-generation reproduction study was recently completed by Hugot et al. (1980). The authors administered sodium nitrite at 1,500 ppm or 3,000 ppm (75 or 150 mg/kg/day) in the diets of groups of 66 Wistar rats. There were no effects on the percentage of matings resulting in pregnancy, the average of implanted embryos, the frequency of resorptions, the percentage of pregnancies resulting in the birth of live litters, the sex ratio, or the percentage of born animals that survived three days. Birth weight was slightly lower in F_{1b} and F_{2b} pups in the group fed 3,000 ppm. After birth, pups of the 1,500 ppm group and particularly the 3,000 ppm group lagged behind the controls in their growth rates. This effect was most prominent in the F_{2a} and F_{2b} groups. In the treated groups, changes in various organ weights were observed in a high proportion of pups at weaning. These included increased liver, kidney and thymus weights. Histological studies were done on F_{2b} pups only. Abnormalities observed in these weanlings included fatty degeneration of the liver, lung lesions, spleen congestion, and thin fur. The authors concluded that the effects of nitrite ingestion on fertility and gestation in the rat were very limited, but that the body and organ growth of the pups between birth and weaning was markedly affected.

The nitrosation of amides or amines in the acid stomach produces N-nitroso compounds which may pass through the placenta to exert teratogenic or fetotoxic effects. Ivankovic et al. (1973) reported the teratogenic effect of oral ethylurea (100 mg/kg) with sodium nitrite (50 mg/kg). A total of 160 offspring were born. Four to six weeks after birth, 96 (60%) of the young animals developed hydrocephalus and died within two weeks. No other malformations were seen. Seventeen additional pregnant Wistar rats were given the same treatment but with ascorbic acid (250 mg/kg) administered orally at the same time. A total of 113 offspring were reared after birth and none showed symptoms of hydrocephalus one year later. This study lacked an ethylurea control so the involvement of nitrite has not been proven.

Teramoto et al. (1980) showed the teratogenicity of ethylenethiourea after its reaction with sodium nitrite in the stomachs of mice. Ethylenethiourea is a decomposition product of ethylenebisdithiocarbamate fungicides. The ethylenebisdithiocarbamates include maneb and zineb, for which residue tolerances on food are as high as 10 ppm and 25 ppm, respectively (40 CFR 180). Ethylenethiourea is reported to be a teratogen by itself in rats and hamsters but not in mice. Ethylenethiourea was administered orally at 400 mg/kg on various days of pregnancy in combination with 50, 100 or 200 mg/kg sodium nitrite at varied intervals. When nitrite was given to mice immediately after their treatment with ethylenethiourea on day six or eight of pregnancy, fetal survival was decreased. Various types of malformations were observed in the living fetuses from dams treated with the two higher doses of nitrite plus ethylenethiourea on days six, eight or ten of pregnancy, but malformations were not observed in fetuses from dams treated on day 12. Teratogenicity was not observed when nitrite was given two hours after ethylenethiourea or by either chemical alone.

D. Mutagenicity

Three methods were used by the U.S. Food and Drug Administration to evaluate the genetic effects of sodium nitrite and sodium nitrate (FDA 1972e, f). They were:

a. Host-mediated assay and *in vitro* assay:
    Mutagenic response in *Salmonella typhimurium*
    Recombination frequency in *Saccharomyces cerevisiae*

b. Cytogenetic assay:
    Rat bone marrow cells
    Human embryonic lung cell line WI-38

c. Dominant lethal gene test in rats

Doses used for the rat tests are shown in Table V-5. Both sodium nitrite and sodium nitrate were negative in the host-mediated mutation assays (mouse strain not given), the cytogenetic assay in Sprague-Dawley rat bone marrow cells, and the dominant lethal gene test in Sprague-Dawley rats. Both increased the number of aberrant WI-38 cells. In addition, sodium nitrite was positive

Table V-5 FDA Mutagenicity Study Protocols

Compound	Dose in mg/kg/day - Rats		
	MTD[a]	Medium	Low
Sodium Nitrite	70	50	30
Sodium Nitrate	1600	800	30
Potassium Nitrite	140	46.7	14
Potassium Nitrate	12	4	1.2

[a] Maximum tolerated dose.

Adapted from FDA 1972 e,f; 1979 a,b.

for mutations in the microorganisms in vitro while sodium nitrate was negative in this assay.

Potassium nitrite and potassium nitrate were tested by the rat dominant lethal mutation test (FDA 1979a, b) and the results were negative. Doses reported to have been used are presented in Table V-5. This part of the report, however, is not credible. The highest dose reported for potassium nitrite is greater than the LD_{50} for sodium nitrite determined in the 1972 FDA study for rats of the size used, yet here the rats are reported to have gained weight faster than controls. The LD_{50} values for nitrates exceed those of nitrites by at least 10-fold for all species reported (Table V-1), yet this study reports doses more than 10-fold lower for nitrate than for nitrite. If the study is reported accurately, the negative result for potassium nitrate is invalid because an inadequate dose was used.

Hussain and Ehrenberg (1974) confirmed the mutagenicity of sodium nitrite to bacterial cells by inducing mutations to streptomycin independence in E. coli Sd-4. Kodama et al. (1976) confirmed the mutagenicity of sodium nitrite to mammalian cells in culture by inducing mutations to 8-azaguanine resistance in cultured FM3A cells, a C3H mouse mammary carcinoma cell line.

Two other host-mediated assays have found no mutagenicity for sodium nitrite. Couch and Friedman (1975) studied Salmonella typhimurium G46 injected intraperitoneally into male ICR mice immediately after gavage with

150 mg/kg sodium nitrite. Whong et al. (1979) studied the same organism injected intravenously into 25-g female CDI mice and 75-g CD rats and recovered (40%) from the liver. Sodium nitrite was given by gavage ten minutes after the test bacteria were injected. The dose was 160 mg/kg for mice and 150 mg/kg for rats.

A transplacental assay (Inui et al. 1980) detected a small increase (3.7 times control) in mutations to 8-azaguanine resistance in Syrian hamster embryo cells cultured 24 hours after gavage of pregnant hamsters with 100 mg/kg sodium nitrite. However, in a parallel experiment hamsters given the same dose were shown to have 40 μg/hamster nitrosodimethylamine in the stomach 30 minutes after gavage.

The above papers by Couch and Friedman (1975), Whong et al. (1979) and Inui et al. (1980) report significant mutagenicity after gavage of four different nitrosatable substrates with sodium nitrite. These results are probably due to the formation of mutagenic N-nitroso compounds in the stomachs of these rodents, rather than direct mutagenicity of sodium nitrate. Two other studies in mice detected mutagenic activity after gavage of nitrite and five other nitrosatable substrates using Salmonella typhimurium injected intraperitoneally or intravenously as genetic indicator (Braun et al. 1977, 1980). The lowest effective dose of sodium nitrite in these five studies was 13.8 mg/kg reported by Braun et al. (1980) using the more sensitive liver site for the tester bacteria. One-tenth of that dose gave no increase in mutation frequency with an equimolar dose of aminophenazone.

E. Carcinogenicity

Greenblatt and Mirvish (1972) administered sodium nitrate in distilled water (12,300 mg/L) to 40 strain A mice as a substitute for drinking water (about 2,050 mg/kg/day). An equal number of mice served as controls for this and other experiments. The animals drank the treated water for 25 weeks. The experiment was terminated after an additional 13 weeks. The number of lung tumors in the treated and control mice was similar. No other tissue was examined and the treatment period was too short for a general carcinogenicity study.

Lijinsky et al. (1973) administered 100 mg of sodium nitrate daily in drinking water (average 238 mg/kg/day) to each of 15 male and 15 female MRC rats. Administration was five days a week for 84 weeks followed by a 20-week observation period. Rats were eight- to ten-weeks-old at the start. An increase in pituitary tumors was observed in treated females (11/15 treated vs. 3/15 controls). The authors state, "The high incidence of tumors of normal type in both experimental and control groups is unexpected and difficult to explain... Our groups of animals were small, and statistical analysis of the results could not be conclusive, though no difference in tumor incidence between groups was deduced" (p. 1062).

Sugiyama et al. (1979) reported an experiment in which groups of 50 ICR mice of each sex were fed either 0, 2,500 or 5,000 mg/kg/day sodium nitrate for more than a year. No increased incidence of tumors was reported. Only the

English abstract and tables have been reviewed and it is not clear whether the data are from necropsy or histopathology. The time period of this study may have been too short to be conclusive.

Most of the data on the carcinogenicity of nitrite come from data on tumor incidence in animals administered nitrite alone in experiments designed primarily to study the carcinogenic effects resulting from the simultaneous administration of nitrite and a nitrosatable substrate.

Garcia and Lijinsky (1973) administered sodium nitrite in drinking water at 0 or 200 mg/L (about 27 mg/kg/day) to 15 MRC rats of each sex for 75 weeks. Animals were kept until death and subjected to complete pathological examination. There were a total of 19 tumor-bearing animals in the treated groups compared to nine in the controls. The only notable specific increase was seven pituitary tumors in treated rats compared to three in controls. This doubling was not statistically significant due to the small test group used.

Taylor and Lijinsky (1975) administered sodium nitrite in drinking water at 200 mg/L (about 27 mg/kg/day) to 27 male and 30 female Sprague-Dawley rats for 104 weeks. Necropsy was performed and tissues from all lesions and major organs were subjected to histological examination. Various endocrine tumors were present (number and type not given). There was no untreated control group.

Inai et al. (1979) administered sodium nitrite at 5,000, 2,500 and 1,250 mg/L in drinking water (about 833, 416, and 208 mg/kg/day) to groups of 50 male and 50 female ICR mice for 18 months. No tumors attributable to nitrite treatment were observed in histopathological examinations of major organs.

Aoyagi et al. (1980) induced liver tumors in 5 of 19 male Wistar rats with 80 mg/kg/day sodium nitrite in food. None of 19 controls had liver tumors. However, nitrosodimethylamine and nitrosopyrrolidine were found in the diet which contained fish meal. This raises questions regarding the validity of the study.

Mirvish et al. (1980) reported that papillomas of the forestomach developed in 8 of 45 MRC Wistar rats (of both sexes). A 3,000 mg/L solution (about 284 mg/kg/day) of sodium nitrite was administered in distilled drinking water for five days/week for life. Two of 91 untreated rats developed these tumors ($P<0.002$). However, these rats were fed Wayne Lab-Blox which contains fish meal, so the validity of this study is also questionable.

Shank and Newberne (1976) reported that 96 Sprague-Dawley rats of both sexes fed sodium nitrite at a level of 50 mg/kg/day had a 27% incidence of tumors of the lymphoreticular system. This was compared to a 6% incidence in 156 untreated control rats. Tumors in organs other than liver were seen in 61% of the treated rats and 18% of the control rats. The diet was an agar-gel semisynthetic diet containing no fish meal. Treatment was begun at the time of conception by feeding the nitrite diet to the dams. No tumors of the lymphoreticular system or any other carcinogenic effect was found in Syrian golden hamsters treated similarly.

In a large lifetime study supported by the Food and Drug Administration, Newberne (1978, 1979) administered sodium nitrite to groups of 68 Sprague-Dawley rats of each sex in various diets (Table V-6) from the time of conception through their natural life span. The rats survived well. The author observed the usual spectrum of neoplastic and non-neoplastic diseases associated with old rats. The tissue changes of most interest were observed in the spleen, lymph nodes, and other components of the lymphoreticular tissues. The spleen exhibited a lesion referred to as immunoblastic cell proliferation. When spleen and non-spleen lymphomas were combined, there were suggestions for a nitrite effect but there was not a convincing dose response. Combining all nitrite-treated groups and all control groups there were incidence rates of 8.5% and 12.5% lymphomas in control and nitrite-treated groups, respectively. Combining both lymphomas and immunoblastic cases of treated and untreated groups the incidence rates of lymphoreticular neoplasia were 15.3% in untreated and 23.7% in nitrite-treated animals.

Newberne's histopathologic assessment of the tissues indicated that, by considering all the groups receiving sodium nitrite together, there was a statistically significant excess of lymphoid tumors ($P<0.01$, based on chi-square analysis). This was reflected especially in the groups receiving sodium nitrite in drinking water.

Table V-6 FDA Carcinogenicity Study Results

			Proportion of rats with:	
Treatment	Group	Nitrite mg/kg/day[a]	Malignant Lymphomas	Immunoblastic cell proliferation
In semipurified agar diet[b]	1	0	5/136	10/136
	2	12.5	10/136	9/136
	3	25	11/136	23/136
	4	50	11/136	14/136
	5	100	14/136	23/136
In water (agar diet)				
0.1%	6	100[c]	16/136	17/136
0.2%	7	200	14/136	18/134
Positive control 2,000 ppm urethane (agar diet)	8		37/135	0/135
Commercial chow diet (Purina)	9	0	9/132	5/132
	10	50	14/134	12/134
	11	100	12/132	11/132
Positive control 2,000 ppm urethane (chow diet)	12		19/136	0/136
Casein diet	13	0	10/136	10/136
	14	50	18/136	11/136
Agar diet (mothers of groups 1 and 4)	15	0	1/33	8/33
	16	50	6/34	8/34
Nitrite exposure after weaning (agar diet)	17	0	6/136	21/136
	18	50	6/131	18/131

[a] Unless otherwise noted.

[b] Ingredients of Agar Diet:

Casein	220
Sucrose	147
Dextrose	197
Dextrin	197
Corn Oil (Mazola)	150
Vitamin Mix	10
Mineral Mix	40
Agar	35
Distilled Water	1,000

[c] Calculated by assuming water consumption of 10% of body weight per day.

Adapted from Newberne 1978, 1979.

After Newberne's report was submitted, a Government Interagency Working Group on Nitrite Research reviewed a sample of histological slides from the study and decided that there was sufficient difference of opinion in the diagnoses to warrant a further evaluation of the histopathological findings. The Universities Associated for Research and Education in Pathology (UAREP), a nonprofit consortium of 15 universities organized to carry out educational and research activities in pathology, was selected by the FDA to review the slides (FDA 1980a). A Joint Committee of Experts was established by the UAREP to perform this review. The committee members diagnosed fewer lymphomas than Newberne had reported. The disparity between the two series of diagnoses involved the differentiation of lymphomas from extramedullary hematopoiesis, plasmacytosis, or histiocytic sarcoma. Furthermore, the committee did not interpret the lesions in the spleen and lymph nodes as immunoblastic hyperplasia as did Newberne. These lesions were interpreted predominantly as extramedullary hematopoiesis and plasmacytosis in the spleen and plasmacytosis or reactive lymphoid hyperplasia in lymph nodes.

In its final report to the FDA, the Government Interagency Working Group summarized the UAREP committee's findings and concluded that most of the lymphoma diagnoses originally reported were not confirmed. The UAREP pathologists diagnosed fewer lesions as lymphoma, with a resulting reduction of incidence to approximately 1% among treated and control groups. This rate of lymphoma incidence is similar to that usually seen spontaneously in Sprague-Dawley rats. UAREP pathologists did report a greater than 1% incidence of other types of tumors, including histiocytic sarcomas. However, no demonstration could be found that the increased incidences of these tumors were induced by the ingestion of sodium nitrite (FDA 1980b).

Maekawa et al. (1982) recently completed a two-year bioassay of the carcinogenicity of sodium nitrite and sodium nitrate in F-344 rats. Maximum tolerated doses determined in a preliminary 6-week study were 0.25% sodium nitrite in drinking water and 5% sodium nitrate in the diet. Groups of 50 males and 50 females were administered the maximum tolerated doses, 50% of the maximum tolerated doses or no nitrate/nitrite (controls). Tumor incidence in all groups was close to 100%, but there were no significant differences in mean survival time, mean time to tumor, increase of malignant tumors or increase of any specific tumor. Other chronic health effects were apparently not looked for.

Twenty-three recent studies demonstrating carcinogenesis in test animals by administering nitrite and nitrosatable substrates orally at appropriate times were reviewed in preparing this report. Some of these studies have been discussed partially in previous sections (Sander and Schweinsberg 1972, Greenblatt and Mirvish 1972, Garcia and Lijinsky 1973, Lijinsky et al. 1973, Taylor and Lijinsky 1975, Shank and Newberne 1976, Mirvish et al. 1980). Also several studies have demonstrated transplacental carcinogenesis by nitrite and suitable substrates administered orally at the sensitive stage of development. If ethylurea and nitrite are fed to rats on the 10th and 11th days of gestation, all progeny die of hydrocephalus (see Section V.C.4. above). If the identical treatment is administered in the last trimester, all progeny die of neurogenic tumors (Ivankovic 1979). Prenatal carcinogenesis is more often associated with direct-acting carcinogens such as nitrosamides and nitrosoureas than with the nitrosamines.

Lijinsky and Rueber (1980) administered 0.2% (100 mg/kg/day) sodium nitrite and 0.1% (50 mg/kg/day) disulfiram (fungicide, prescription drug and rubber accelerator) in a powdered diet to 40 Fischer 344 rats. Rats were eight- to nine-weeks-old at the start of treatment which continued for 78 weeks followed by observation until natural death. This treatment induced tumors of the stomach, esophagus, tongue, and nasal cavity in 10 of 20 male and 12 of 20 female rats. None of these tumors was found in rats administered either chemical alone. The nitrosation product of disulfiram is nitrosodiethylamine.

Taylor and Lijinsky (1975) administered 0.2% sodium nitrite (133 mg/kg/day) and 0.2% heptamethyleneimine (133 mg/kg/day) in drinking water to 30 Sprague-Dawley rats fed Purina lab chow. Rats were eight- to ten-weeks-old at the start and were treated for 28 weeks and observed until death. This treatment induced tumors of the oropharynx, tongue, forestomach or esophagus in 25 of 30 rats (15 of each sex). Fifty-seven nitrite-treated controls had no tumors in the above tissues.

Over 130 N-nitroso compounds have been tested for carcinogenicity. Greater than 120 of them have been shown to be carcinogenic (Shank and Magee 1981). Diethylnitrosamine has been shown to be carcinogenic in at least 22 species and transplacentally carcinogenic in at least five species (Schmahl and Habs 1980). All species tested have shown tumor formation following treatment with one or more N-nitroso compounds. Tumors have been induced in every organ and tissue and from most types of cells following treatment of animals with some nitroso compound. The responses show organ specificity within a species, even after administration by different routes. However, striking differences in target tissue are seen between species (Lijinsky et al. 1975).

Substrates which can be nitrosated include commonly used drugs, pesticides, industrial chemicals and natural products (Montesano and Bartsch 1976). Quarles et al. (1979) used the transplacental host-mediated hamster cell culture assay to demonstrate the carcinogenicity of N-nitroso derivatives of the pesticides carbaryl, carbofuran, Baygon,[a] Landrin,[a] Bux Ten,[a] methomyl and aldicarb, none of which was active in this assay without nitrosation. Test compounds were injected intraperitoneally on the tenth day of gestation, and fetal cell cultures were prepared on the 13th day. Cultures were studied through ten subcultures (20 cell doublings) and transformation was confirmed by growth in soft agar and by tumor formation in nude (athymic) mice. Six of these nitrosatable pesticides are allowed as residues on food.

Reaction of nitrite with the amino acids used in protein synthesis has not produced carcinogenic products (Greenblatt et al. 1973).

As in acute toxicity, teratogenesis and mutagenesis by N-nitroso compounds formed from nitrite and substrates in the acid stomach, adult and transplacental carcinogenesis by this mechanism can also be blocked by the natural antioxidant ascorbic acid (Akin and Wasserman 1975, Rustia 1975, Ivankovic et al. 1975,

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[a] Registered trademark.

Mirvish et al. 1976). However, when the latter authors tested the ability of sodium ascorbate to block nitrosation in groups of 40 eight-week-old male MRC-Wistar rats, the target organ changed from liver to forestomach. Group I was treated with 3 g/L sodium nitrite in drinking water (about 400 mg/kg/day) and 500 mg/kg/day morpholine in food. Group II was administered the same treatment plus 1,135 mg/kg/day sodium ascorbate in food. The control group was kept on normal diet and plain water. Liver tumors appeared in 65% of group I, 49% of group II, and none of the controls. Forestomach tumors appeared in none of group I, 54% of group II, and 4% of untreated animals.

F. Summary

The acute lethal doses of nitrate and nitrite vary widely between species, but that of nitrate is generally more than ten times as high as that of nitrite. The toxicity of nitrate is due to its reduction to nitrite by bacteria. Therefore, oral intake of nitrate is more toxic in ruminants than in monogastric animals. Some species such as dogs and pigs do not convert nitrate to nitrite sufficiently to cause toxicity.

Acute toxic effects of nitrite are manifested as methemoglobinemia and circulatory collapse. Which of these predominates in a particular species depends on how rapidly nitrite is removed from blood plasma by the formation of methemoglobin and nitrate. Toxic vasodilation occurs only in those species with slower rates of methemoglobin formation, especially the horse. Reported chronic effects of high nitrate intake include diuretic effect, depletion of vitamins A and E, depression of motor activity, brain electrical changes, and histological changes in the heart, lungs, liver and spleen.

Sodium and potassium nitrates and nitrites tested in four species of animals did not cause teratogenic effects. The nitrites did cause delay in the skeletal maturation in rats and hamsters. Fetal methemoglobinemia and death could be produced by administration of nitrite to pregnant rats and guinea pigs. A three-generation reproduction test in rats showed no prenatal problems. After birth, growth was retarded and weanlings showed histological abnormalities of the liver, lungs and spleen. The oral administration of nitrite with ethylenethiourea produced teratogenic effects early in pregnancy and transplacental carcinogenesis later.

Sodium nitrate and nitrite were tested for mutagenicity by several methods. Both were negative in all in vivo assays; however, sodium nitrite was positive in in vitro assays performed by two laboratories. Gavage of rats with nitrate and nitrosatable compounds produced mutagens in the gastric contents and mutagenic activity in host-mediated bacterial systems.

Assays of the carcinogenicity of nitrate have been negative. Liver and stomach tumors were induced in rats administered sodium nitrite in food or water. In both positive studies, animals were fed diets containing fish meal, which is highly nitrosatable in the stomach. Other studies on the carcinogenicity of nitrite were negative except for one laboratory in which significant increases in tumors of the lymphoreticular system were reported in rats fed sodium nitrite. Review of the slides from these studies by other pathologists resulted in segregation of a number of histiocytic sarcomas from the lymphoma group resulting in lack of statistical significance for either group and the conclusion that nitrite was non-carcinogenic.

Many studies have documented carcinogenesis (adult and prenatal) in animals given oral nitrite and nitrosatable compounds. Tumors induced were in many organs including the stomach, esophagus and nasal cavity. Carcinogenesis in several of these experiments could be blocked by simultaneous oral administration of ascorbic acid. In one study, liver tumors were decreased by ascorbate, but forestomach tumors developed instead.

VI. HEALTH EFFECTS IN HUMANS

A. Acute Toxicity

The toxicity of nitrate in humans is thought to be due to its reduction to nitrite in saliva. The lethal dose of potassium nitrate for an adult ranges from 54 mg/kg to 462 mg/kg. The lethal dose of sodium nitrite ranges from 32 mg/kg to 154 mg/kg (Burden 1961).

1. Cardiovascular Effect

An experiment in human acute toxicity was reported by Weiss et al. (1937). A normal male was given 0.18 g (about 2.6 mg/kg) sodium nitrite orally. Only minor changes were recorded while he remained in the horizontal position. When he was tilted to a 75° upright position his condition began to change rapidly. Yawning appeared and became progressively more prominent. The subject's respiration deepened and assumed a sighing character, restlessness and belching developed, and a cold sweat appeared on the face and extremities and spread over the entire body. The pulse pressure became very narrow as a result of a fall in systolic pressure and a significant rise in diastolic pressure. The central venous pressure fell progressively to well below the hydrostatic level of the right atrium. Blood flow in the hands decreased and the heart rate increased as soon as tilting occurred; both fell precipitously just before loss of consciousness. The subject soon developed the clinical picture of cardiovascular collapse. He was drowsy, the skin was ashen-gray and the pupils were dilated. Vision became dim, muscle power was lost and unconsciousness ensued. The subject was immediately returned to the horizontal position and regained consciousness within 20 seconds. All circulatory abnormalities promptly disappeared. This sequence of events was confirmed in six other young adults (Weiss et al. 1937).

Severe poisoning by nitrates was reported by Harris et al. (1979). Three men were involved in an industrial accident in which a chamber containing 50% sodium nitrate and 50% potassium nitrate at 246°C exploded, spraying the workers with molten chemicals. Their clothing was immediately removed and the men were taken to a hospital for treatment of extensive burns. Within 15 to 20 minutes, all three were cyanotic and two had generalized seizures. Cyanosis was not corrected by oxygen therapy and the men's methemoglobin levels were determined to be 65%, 56% and 42%. A few minutes later one man suffered a cardiac arrest and was unresponsive to all resuscitation measures. The other two men were treated with methylene blue antidote but remained cyanotic and so were transfused with 22 and 15 units of whole blood while the white chemical crust was cleaned off their skin. Methemoglobin levels were normal at the end of the exchange transfusions and the two men recovered.

Acute toxicity from sodium nitrite ingestion was recently described by Aquanno et al. (1981). Two laboratory technologists salted their breakfast with a laboratory salt shaker containing sodium nitrite. Symptoms appearing within 30 minutes included weakness, sweating, nausea, throbbing and a roaring sound in the ears, palpitations, numbness and tingling. Both went to the emergency room for treatment and were found to have cyanosis with methemoglobin levels of 34% and 54%. Treatment with the antidote methylene blue lowered methemoglobin levels to normal in one hour.

2. Effect on Hemoglobin

Comly (1945) first reported methemoglobinemia in two infants which could be traced to making formula with water containing ≥90 mg/L nitrate-nitrogen. Donahoe (1949) reported five cases of methemoglobinemia in infants less than three-months-old. All cases could be traced to well water contaminated with nitrate. Cyanosis appeared within a few days to two weeks of feeding formula made with the contaminated water. In two cases the nitrate concentration of the contaminated water was measured to be 50 and 177 mg/L. This is equal to 11.3 and 40 mg/L nitrate-nitrogen. Coliform organisms were also present in the contaminated water. Thus, nitrite may have been present also. A survey by the American Public Health Association found over 278 cases of methemoglobinemia, including 39 deaths, definitely associated with nitrate-contaminated water (Walton 1951). This study is summarized in Table VI-1. No cases associated with water containing 10 mg/L or less of nitrate-nitrogen were found.

By 1970, about 2,000 cases of infant methemoglobinemia (including many fatal poisonings) resulting from the consumption of water with high nitrate concentrations had been recorded in various countries throughout the world (Shuval and Gruener 1972).

Table VI-1 Infant Methemoglobinemia and Nitrate Concentrations in Drinking Water

Relation to Nitrate-Nitrogen Concentration of Water Used in Feeding Formula								
	Methemoglobinemia		Number of cases associated with indicated ranges of NO_3^--N concentration (ppm)					
State	Reported cases	Reported deaths	0-10	11-20	21-30	31-50	51-100	100+
California	1	0	0	0	0	0	1	0
Georgia	6	3	-	-	-	-	-	-
Illinois	75	6	0	1	2	2	12	11
Indiana	1	0	0	0	0	0	1	0
Iowa	Several	11	0	0	0	0	1	1
Kansas	13	3	0	0	1	1	2	8
Michigan	7	0	0	0	0	0	0	7
Minnesota	139	14	0	2	25		53	49
Missouri	2	0	0	0	0	0	0	2
Nebraska	22	1	0	1	0	4	9	8
New York	2	0	0	0	0	0	1	0
North Dakota	9	1	0	1	1	0	0	6
Ohio	0	0	0	0	0	0	0	0
Oklahoma	0	0	0	0	0	0	0	0
South Dakota	Several	0	-	-	-	-	-	-
Texas	0	0	0	0	0	0	0	0
Virginia	1	0	0	0	0	0	1	0
Total	278+	39	0	5	36[a]		81	92
Percent of Total			0.0	2.3	16.8		37.8	43.1

[a] Number of cases in range 21-50 ppm

Adapted from Walton 1951

In a controlled experiment in a hospital, 104 infants were fed powdered formula (Gruener and Toeplitz 1975). All infants were initially given formula made with water containing 15 mg/L nitrate ion (i.e., NO_3^-). They were then fed formula mixed with water containing 108 mg/L nitrate ion. There was a rise in methemoglobin levels in most infants after the first day of exposure to high nitrate formula. The infants remained on the high nitrate formula for three days. There was a drop in methemoglobin to almost normal levels on the second day of exposure to high nitrate formula, suggesting some adaptation. The starting methemoglobin level in this study averaged 0.9%. The higher levels averaged 1.3%. No clinical methemoglobinemia developed, although three infants (age not given) reached second day methemoglobin levels of 6.9%, 13.9% and 15.9%.

Winton et al. (1971) studied a group of 111 infants in which 63 received a nitrate dose of less than 1 mg/kg/day, 23 were exposed to 1-4.9 mg/kg/day, 20 were exposed to 5.0-9.9 mg/kg/day, and 5 infants were exposed to 10-15.5 mg/kg/day. Only three infants appeared to have methemoglobin levels above normal (0-2.9%) and they were the youngest of the five who had received more than 10 mg/kg/day. The highest methemoglobin level (5.3%) was found in a 30-day-old baby who had received 15.5 mg/kg/day.

In the Federal Republic of Germany, Simon et al. (1964) reported results of a survey of 745 cases of infant methemoglobinemia. Nitrate concentrations in the water were less than 100 mg/L in 16.2% of the cases. Nitrite was found in 10.4% of the water samples. Of the infants surveyed 97.7% were less than than 3 months, 53% had diarrhea and 8.6% died. Infant formulas were powdered milk in approximately one-third of the cases. The remaining two-thirds received either diluted cow's milk or an unspecified diet. The unspecified diet was administered to those infants with diarrhea, presumably 53% of all the cases. However, the authors did not specify the exact number of cases receiving each diet. Healthly infants aged one to three months showed methemoglobin concentrations in their blood proportional to the nitrate content of their drinking water. Infants older than three months had low values of methemoglobin which were independent of the nitrate concentration in the drinking water.

Toussaint and Wurkert (1982) studied the methemoglobin levels of 68 infants in Germany as a function of the nitrate concentration in drinking water. They found mean methemoglobin levels below 3% at nitrate concentrations below 50 mg/L, with methemoglobin levels increasing with nitrate dose. In areas with drinking water nitrate below 30 mg/L, the authors detected that infant methemoglobin levels increased when the diet was supplemented with tea or vegetables or when the infant had an infection.

Shearer et al. (1972) studied 256 infants up to six months of age in southern California. No clinical methemoglobinemia was found but methemoglobin levels over 3% were observed in about 30% of the infants under two months of age and in about 10% of those in the third month, independent of nitrate ingestion. Methemoglobin levels greater than 4% were observed in 4% of the infants under 3-months-old whose nitrate-nitrogen intake was less than 5 mg/day (equivalent to a powdered formula with water containing less than 6.7 mg/L nitrate-nitrogen). Methemoglobin levels greater than 4% were also observed in 14% of these infants ingesting between 5 and 10 mg nitrate-nitrogen per day. Methemoglobin levels over 6% were observed only in young infants with mild respiratory infections or diarrhea. One infant hospitalized with diarrhea had a methemoglobin level of 8.4% although the child had been consistently receiving bottled water.

Hegesh and Shiloah (1982) studied the relationship of methemoglobinemia to nitrate or nitrite ingestion in hospitalized infants with diarrhea. Fifty-eight infants with diarrhea and 130 controls of different ages without gastro-intestinal disturbances were compared as to blood nitrate concentration and methemoglobin level. Sixty-nine percent of the infants with diarrhea had methemoglobin levels above those for the infants without diarrhea. The authors demonstrated that the infants with diarrhea excreted up to 10 times more nitrate than they ingested, while the controls excreted approximately 2.5 times their nitrate intake. No correlation between blood nitrate level and nitrate ingestion was found in the infants with diarrhea, with the authors concluding that diarrhea alone can cause infant methemoglobinemia.

Craun et al. (1981) conducted an epidemiologic study of 102 children aged one to eight years in Washington County, Illinois. A study of this area showed that 50% of the private wells tested had nitrate levels above 22 mg/L nitrate-nitrogen. Sixty-four of the children were selected from familes consuming high-nitrate water (22-111 mg/L nitrate-nitrogen) and 38 children (controls) were from families consuming water containing less than 10 mg/L nitrate-nitrogen. The study did not show that ingestion of water with a nitrate concentration of 22 to 111 mg/L nitrate-nitrogen was related to increasing methemoglobin levels nor that the children had above-normal methemoglobin levels. The only correlation found was the known decrease in methemoglobin level with increase in age.

Super et al. (1981) studied 486 infants from one to twelve months of age in southwest Africa. Of these infants, 318 had been fed formula or cow or goat milk in addition to or in place of breast milk. Methemoglobin values and drinking water nitrate intake were determined. There was a highly significant correlation ($P<0.01$) between these two parameters in all six age groups studied, and no significant relationship ($P>0.05$) between age and methemoglobin level. Vitamin C supplementation had a significant effect ($P\leq0.03$) on methemoglobin levels in high nitrate regions (>20 mg/L nitrate-nitrogen). Here, 38 of 104 infants not given vitamin C had methemoglobin levels above 3.0% while only 1 of 18 infants with daily vitamin C supplements was observed to have a methemoglobin level above 3%.

As a general rule, older children and adults are not susceptible to methemoglobinemia from water nitrate levels which adversely affect infants. However, Petukhov et al. (1972) observed symptoms at all ages in Russia. The authors investigated the nitrate content of well water in three settlements and its effects on the health of 453 persons, aged six months to 75 years. When the concentration of nitrate-nitrogen in the water was from 23.7 to 44.6 mg/L (equivalent to 105-198 mg/L nitrate ion), methemoglobinemia was found to develop in both children and adults. Cyanosis, a fall in blood pressure and an increase of the methemoglobin content of blood were noted in all of the sick persons. A rise of the pulse rate was noted in children under six years of age and an increase of the respiration rate was noted in children under 13 years of age.

These observations in children are in agreement with the studies of Metcalf (1961) that increased sensitivity to methemoglobin formation is present in children of both sexes up to the age of puberty. Metcalf (1961) also observed increased sensitivity of hemoglobin to oxidation by nitrite in pregnant women and patients with cancer. Blood samples from 100 pregnant women, 51 cancer patients, and 100 medical and physiotherapy students were tested. Increased sensitivity of the hemoglobin to oxidation was exhibited in 96% of women more than six weeks pregnant. A similar but less marked change occurred in the patients with cancer. No cases of increased sensitivity occurred in any of the students. In pregnant women, the sensitivity of the hemoglobin to oxidation rapidly returned to normal after delivery. In the few cancer patients examined, the sensitivity of hemoglobin returned to normal following complete surgical removal of the malignant tumor.

Individuals with altered physiological states or with either hereditary or acquired disease may also be predisposed to the development of nitrite- or nitrate-induced methemoglobinemia. These include individuals with glucose-6-phosphate dehydrogenase deficiency, adults with reduced gastric acidity (including those being treated for peptic ulcer or individuals with chronic gastritis or pernicious anemia) and individuals with a rare, hereditary lack of NADH or methemoglobin reductase activity in their red blood cells (Scott 1960). This hereditary enzyme deficiency seems also to account for the somewhat increased incidence of methemoglobinemia among Alaskan Eskimos and Indians (Scott and Hoskins 1958). Some Caucasian ethnic groups, including Sardinians, Sephardic Jews, Greeks and Iranians have an erythrocyte glucose-6-phosphate dehydrogenase deficiency. A similar deficiency has been demonstrated in approximately 13% of black males in Illinois (Tarlov et al. 1962). These individuals are less able to reduce methemoglobin to hemoglobin in response to the nitrite antidote, methylene blue (see Figure VII-1).

B. Chronic Toxicity

Some evidence that chronic use of high-nitrate drinking water may directly affect the central nervous system in humans was provided by Petukhov and Ivanov (1970). The authors described the slowing of conditioned motor reflexes in response to auditory and visual stimuli in 39 Russian children whose drinking water contained nitrate at 105 mg/L. Their reflexes were compared with those of a group of children whose drinking water contained 8 mg/L. Children drinking the high-nitrate water had average methemoglobin levels of 5.3%.

Moneret-Vautrin et al. (1980) reported a syndrome of chronic urticaria and repetitive vasomotor headache which could be cured or greatly improved by removing added nitrite from the diet. The symptoms were reproducible in blind provocative testing. The toxic effect is not authentic food allergy, but seems to involve an interaction with pork in the gastro-intestinal tract. Hypotheses are suggested by the authors for this interaction.

C. Teratogenicity and Reproductive Effects

Scragg et al. (1982) studied birth defect incidence in relation to nitrate content of the household water supply in the Mount Gambier region of South Australia. This case-control study showed a relative risk of malformation of 2.8 for infants of women drinking lake water (approximately 15 mg/L nitrate) or well water (greater than 15 mg/L nitrate) compared to women drinking rain water (<5 mg/L nitrate). When the analysis was limited to only those women using private wells, the relative risk rose to 4.1. Malformations with the greatest increase in risk for those using groundwater were central nervous system defects (relative risk of 3.5) and multiple system malformations (relative risk of 2.3). The latter number, however, was not statistically significant. Since groundwater contaminated with nitrate is likely to be contaminated with other pollutants, the above results cannot be interpreted to prove that nitrate is teratogenic.

Reports of severe methemoglobinemia in infancy do not mention any infant born with methemoglobinemia. This suggests that even if the mother is drinking high-nitrate water, insufficient nitrite reaches the fetus to produce cyanosis. Nitrate would not be reduced to nitrite in the fetus because of the absence of bacteria in the gastrointestinal tract. Another possible explanation for the lack of reports of methemoglobinemia at birth is that cyanosis of the newborn is so common that a slight additional effect of methemoglobinemia would not be noticed.

Premature infants are reported to be born with higher levels of methemoglobin than full-term infants (Kravitz et al. 1956). Methemoglobin levels in newborn premature infants averaged 2.3% of hemoglobin, while in mature newborns it averaged 1.5%. None of the premature infants studied showed cyanosis.

D. Mutagenicity

Studies attempting to relate human mutations to exposure to nitrates or nitrites were not found. However, studies have shown *in vitro* mutagenicity of human gastric contents with the mutagens identified as nitrosation products of prescription drugs. Stemmermann et al. (1981) detected direct-acting mutagens in mucosa removed from the human stomach during surgery. The mutagens were later identified as the N-nitroso derivatives of three drugs that had been administered to the patients. The drugs were hydroxyzine hydrochloride, diazepam and cimetidine.

A Japanese mackerel fish product treated with excess nitrite at pH 3 was found to contain a direct-acting mutagen, which was not nitrosomethylurea (Mower and Weisburger 1978). The mutagen was not identified.

E. Carcinogenicity

No clinical reports were found in the literature which suggest a relationship between human cancer and exposure to nitrates and nitrites. The International Agency for Cancer Research (IARC) has not evaluated the carcinogenic potential of nitrate, nitrite or N-nitroso compounds (WHO 1982). However, a few laboratory studies have related epidemiological findings to nitrite-nitrosamine exposure.

Nasopharyngeal cancer has been associated with exposure to N-nitroso compounds or their precursors. Some nitrosamines (e.g., nitrosodimethylamine and nitrosodiethylamine) administered at low doses induce tumors of the nasal cavity in rats (Druckrey et al. 1969). Nasopharyngeal cancer is rare in the United States but is common among the Chinese in parts of Southeast Asia, including Hong Kong and Singapore. It may be relevant that nitrosodimethylamine has been detected in salted fish from Hong Kong, although the levels were only 1-35 µg/kg (Huang et al. 1978a). When tested in animals, the same fish product induced tumors of the nasal cavity in four of 20 rats (Huang et al. 1978b).

Japan has the highest rate of gastric cancer in the world. Laboratory studies lend some support to the suggestion that salted dried fish plays a role in the etiology of gastric cancer. For example, treatment of a Japanese dried bonito fish product with excess nitrite produced the carcinogen nitrosomethylurea (Mirvish and Cairnes 1981). The unidentified mutagen produced by treating Japanese mackerel fish with nitrite was fed to rats (12- to 50-day-old Wistar strain) by gavage three times a week for six months. It produced glandular stomach tumors in five of 12 animals and forestomach tumors in two others. Eight control rats had no tumors (Weisburger et al. 1980).

Schmahl and Habs (1980) reviewed the carcinogenicity of N-nitroso compounds. They noted that nitrosomethylurea led exclusively to squamous cell carcinomas of the forestomach after oral application to rats. However, when the compound was given intravenously it caused brain tumors instead.

Organotropic effects of nitrosamines differ from those of direct-acting nitrosamides not only between species, but also with dose within the same species; however, there is little or no variation in organotropism based on route of administration (Schmahl and Habs 1980). Therefore, these authors caution that predictions as to the organotropism of the carcinogenic action in humans cannot be made. It is not known how the organotropism of a substance would differ between animal species if the animals were kept alive for a sufficiently long period to give all of the affected tissues the opportunity to develop a visible tumor.

Species differences in target organ for a particular N-nitroso compound may be only a matter of timing. The animal dies of the tumor that grows fastest, which would preclude development of slower-growing tumors in other tissues. Sometimes a secondary target organ can be identified by using a low enough dose so that not all animals are affected in the primary target organ. Administration of 30 mg/kg nitrosodimethylamine intraperitoneally one time to six 120-g Wistar rats resulted in kidney tumors in two animals seven and ten months after treatment, lung adenocarcinoma in a third at 14 months and malignant lymphomas in the other three 20 to 23 months after treatment (Shearer 1979).

F. Epidemiological Studies

The epidemiology of the relationship between nitrates, nitrites, nitrosamine formation and human cancer has recently been reviewed thoroughly by the Committee on Nitrite and Alternative Curing Agents in Food of the National Research Council, National Academy of Sciences. The following discussion is taken from the Committee's report (NAS 1981).

Stomach cancer incidence shows a geographic distribution. This suggests an environmental factor. Several studies have measured the relationship between nitrate or nitrite intake and stomach cancer.

Colombia has one of the highest rates of mortality from stomach cancer, especially among persons living at high altitudes in certain rural areas (Haenszel and Correa 1975). Correa et al. (1975) have shown geographic correlations of stomach cancer incidence not only with nitrate content of well water but also with the prevalence of atrophic gastritis and to some extent with the nitrate in urine and saliva. In carefully conducted studies involving gastroscopy of volunteers (Correa et al. 1976), they found three-fourths of the high-risk population to have superficial gastritis or other dysplasias. In contrast, a much lower frequency was found in persons from the lower-risk areas of Colombia.

Correa and his colleagues have suggested that superficial gastritis, atrophic gastritis, and intestinal metaplasia are precursor lesions for gastric cancer. They described in detail the histopathology of the gastric lesions, beginning with the normal mucosa and progressing through various degrees of dysplasia to stomach cancer. Analyses of gastric fluid of individuals with atrophic gastritis indicated that nitrite levels were elevated in patients whose gastric pH was above five (Correa et al. 1979). Since nitrosation reactions could occur more readily at higher nitrite concentrations, the formation of N-nitroso compounds may be a key factor in the development of gastric cancer. Data from case-control studies of persons with dysplasia indicate that a history of a high-corn diet is associated with dysplasia. Ingestion of lettuce, which contains ascorbic acid (vitamin C), is inversely associated. Additional data supporting the role of N-nitroso compounds in gastric cancer were reported by Montes et al. (1979) who detected a direct-acting mutagen, possibly a nitrosamide, in the gastric juice of fasting subjects from an area with a high incidence of gastric cancer. Hawksworth et al. (1975) observed concentrations of nitrate as high as 146 mg/L in water supplies serving three areas of Colombia with high rates of stomach cancer.

Chile has natural deposits of nitrate and for decades has used large amounts of these compounds in fertilizers. Gastric cancer mortality in 1960, 1962 and 1964 was statistically correlated with the use of nitrate fertilizers from 1960 to 1964 for each Chilean province (Zaldivar 1977, Zaldivar and Wetterstrand 1975). An association was found between mortality from stomach cancer and the proportion of farmers using nitrate-containing fertilizers in each province; in contrast, there was no association with miners. This association remained significant when data from the industrialized provinces were excluded in order to eliminate the interference of occupational or industrial factors.

A more detailed study was conducted by Armijo and Coulson (1975) from 1945 to 1972. These investigators found no association between stomach cancer and exposure to nitrate in drinking water, but they noted that the levels of nitrate throughout Chile were well below 100 mg/L. However, they did report a high correlation between the gastric cancer mortality in each province and the estimated per capita exposure to nitrogen from fertilizers.

Armijo et al. (1981) interviewed 389 gastric cancer patients and 845 controls at the gastroenterology clinics of seven hospitals in Santiago, Chile. They found that the patients had resided in high-risk areas during their early lives for longer periods than had the controls. Gastric cancer was also found to be associated with previous occupation in agriculture.

In an epidemiological study conducted in England, Hill et al. (1973) correlated differences in stomach cancer mortality rates with the nitrate content of drinking water in two towns. They found that the town with the higher level of nitrate (average, 90 mg/L) had the higher mortality from this cancer. The ratios of observed to expected gastric cancer cases were 1.3 for males and 1.9 for females. Hill and co-workers, however, did not consider socio-economic status, which is known to be inversely correlated with the risk for developing gastric cancer. Davies (1980) suggested that the higher stomach cancer mortality was not related to nitrate intake from drinking water but, rather, to the lower socio-economic status of the inhabitants of the town, which was a mining community.

An epidemiological study (using census data) of workers in the production of nitrate fertilizers is in progress in England (Fraser et al. 1980). Among the 47 deaths which have occurred in a sample of male fertilizer workers between 1971 and 1977, they have observed a deficit of total deaths and deaths from circulatory diseases. They have observed an excess of cancer deaths (SMR 134) which cannot be attributed entirely to regional differences in mortality nor dismissed as a social class effect. Conspicuous elevations were in lung cancer (SMR 152) and gastrointestinal cancer (SMR 169) but the numbers are so small that the difference is not statistically significant (P value not reported). There appears to be a dose-response relationship between total cancer mortality and the frequency of exposure to nitrate-containing dust.

The above trends are consistent with the results of a proportional mortality study within a large fertilizer manufacturing company between 1950 and 1977. However, the number of deaths involved at the time of writing was too small to draw a firm conclusion. The authors (Fraser et al. 1980) stated that they expect to get a much larger volume of data from the 1961 census, which should provide sufficient information to confirm or disprove the preliminary observations.

Amadori et al. (1980) reported an epidemiological study of gastric cancer in Italy. Variables studied were location (rural vs. urban), nitrate level in drinking water, salivary nitrate and nitrite levels, and histological findings in gastric biopsies. The results show a high risk for gastric cancer in the rural area where well water nitrate averaged 38.7 mg/L. Chronic atrophic gastritis was shown in 40 of 46 gastric biopsies in the rural area; none were performed elsewhere. The authors suggest a relationship between high nitrate exposure and high exposure to nitrosatable fungicides of the alkyldithiocarbamate class. These have been shown to nitrosate to nitrosodimethylamine or nitrosodiethylamine (Sen et al. 1974, Lijinsky et al. 1972).

Juhasz et al. (1980) compared stomach cancer incidence in 230 localities in the County of Szabolcs-Szatmar, Hungary, to high or low nitrate content of the local drinking water. High nitrate was defined as greater than 100 mg/L nitrate. An overall correlation with nitrate exposure was found, but many communities did not fit the hypothesis. It was concluded that other factors caused stomach cancer in the low-nitrate areas (not very low by U.S. standards). The authors also concluded that urinary nitrate excretion was a better indicator of gastric cancer risk than the nitrate concentration in the drinking water.

Japan has the highest reported incidence of stomach cancer in the world. Several explanations have been proposed, including an association with the consumption of salted, dried fish products known to contain high levels of certain secondary amines and other precursors of N-nitroso compounds. Salt has been suggested as a promoting factor for this disease (Haenszel and Correa 1975, Sato et al. 1959) and has been demonstrated to be a co-carcinogen in test animals (Kinosita 1969, Tatematsu et al. 1976). Japanese who immigrate to Hawaii continue to be at high risk for stomach cancer, but the risk is substantially lower for the next generation.

Epidemiological studies of these immigrants indicate an association between a high incidence of stomach cancer and the consumption of salted dried fish and salt-pickled vegetables (Haenszel and Correa 1975). In Japan, this association was not confirmed, but investigators reported an elevated risk for stomach cancer among Japanese farmers who consume both types of food more frequently than do other population groups (Haenszel et al. 1976). Other important sources of N-nitroso compound precursors were not ruled out. For example, Japanese farmers, more often than other occupational groups in that country, drink well water that could contain elevated levels of nitrate. They also consume fewer fruits and vegetables containing vitamin C. Vitamin C intake has been hypothesized to be inversely correlated with the incidence of gastric cancer because of its inhibitory effect on nitrosation reactions. Kolonel et al. (1981) have also reported a negative association between fresh fruit consumption and gastric cancer in a prospective study conducted in Hawaii. The study population included Japanese immigrants.

In contrast to the studies linking stomach cancer to nitrate or nitrite exposure, there is no evidence linking large bowel cancer to such exposures (Haenszel et al. 1973).

Esophageal cancer is especially common in parts of Iran, China, the USSR and South Africa. Epidemiological studies conducted in two of these high-incidence areas are described below.

The high incidence of esophageal cancer in the Caspian Littoral of Iran has been studied by a Joint Iran-International Agency for Research on Cancer

Study Group (1977). Villages with different rates of esophageal cancer were surveyed for dietary, work and personal habits. Their main foodstuffs were analyzed for volatile nitrosamines, nitrate, nitrite and other compounds. Their drinking water was tested for nitrate and nitrite.

The average daily intake of nitrate and nitrite was not significantly different for high- and low-incidence areas. For example, a comparison of the nitrate and nitrite content of water showed no elevation in areas at high risk for esophageal cancer. In contrast to these findings concerning nitrate and nitrite in water, Eisenbrand et al. (1980) stated that there were intermittently high levels of nitrite in the saliva of children in the high-incidence area of Iran. This occurred especially on hot days when water intake may not have been sufficient.

The study group also found that in the regions where esophageal cancer was most common, the diet was limited largely to bread and tea, and was low in vitamin C, vitamin A and riboflavin. In a subsequent case-control study in this region, Cook-Mozaffari et al. (1979) also found low intakes of uncooked vegetables (as well as fruits) in cases compared with controls. However, no association was found between the intake of preformed volatile nitrosamines and a high risk of esophageal cancer (Joint Iran-International Agency for Research on Cancer Study Group, 1977). The results of this investigation did not implicate nitrate, nitrite or N-nitroso compounds.

Chinese scientists reported a multifaceted study of esophageal cancer in their country (Coordinating Group for Research on the Etiology of Esophageal

Cancer of North China, 1974). They suggested that nitrosamines are likely to be the causative agents. The mortality from esophageal cancer was high in Linxian (county) in Honan province (111 per 100,000 population), where there is also a high prevalence of esophageal dysplasia. The occurrence of squamous cell carcinoma in the gullet (esophagus) of domestic chickens in the same area is intriguing (176 per 100,000 chickens). This is compared to Fanxian of Honan province which has an esophageal cancer mortality rate of 17 per 100,000 population and an incidence in chickens of 17 per 100,000 chickens. The diet and gastric juices of noncancer patients in Linxian Peoples Hospital have been found to contain secondary amines, nitrate, nitrite and nitrosamines. The diet of this population includes nitrite-rich pickled vegetables and moldy corn bread and is low in riboflavin and seasonally low in carotene and ascorbic acid. The Chinese have begun a preventive effort, discouraging the use of pickled vegetables and fungus-infected corn bread and encouraging the consumption of foods containing ascorbic acid (Yang 1980).

Several studies have demonstrated a correlation between cancer incidence and deficiency of the vitamins which inhibit nitrosation. Meinsma (1964) interviewed 340 stomach cancer patients and a comparable group of other hospital patients (controls) in the Netherlands and noted that the intake of citrus fruits by cancer patients was lower than that of controls.

Mettlin et al. (1981) computed indices for the intake of vitamins A and C in male esophageal cancer patients and controls. They found an inverse correlation between intake of these vitamins, especially vitamin C, and cancer risk.

Armstrong (1980) reported that people in regions of China where stomach cancer is common tend to eat small amounts of fresh fruits and vegetables, meat and eggs. These people do consume large amounts of salted and pickled vegetables and sweet potatoes.

Higginson (1966) compared diets of 93 stomach cancer patients to controls. Stomach cancer patients had a history of a slightly lower consumption of dairy products and fresh fruits. However, the differences between groups were not statistically significant.

Haenszel and Correa (1975) reported three correlations from a case-control study in Minnesota: (1) recent use of cooked cereals, smoked fish and canned fruits was higher and intake of lettuce and tomatoes was lower among stomach cancer patients than among controls; (2) while total intakes of cereal products and fish were only slightly higher than among the controls, the index for total vegetables was considerably lower and the vitamin C index was moderately lower among the stomach cancer patients; and (3) the lower vegetable and vitamin C intake among stomach cancer patients had persisted over a long period.

G. Summary

Poisoning by nitrate or nitrite in humans is evidenced by the cyanosis and anoxia of methemoglobinemia and by symptoms of cardiovascular failure resulting from vasodilation.

Infants are particularly susceptible to methemoglobinemia, especially in the first few months of life. Many incidents of such poisoning have been reported in areas where the nitrate content in water is high (above 20 mg/L nitrate-nitrogen). Water nitrate methemoglobinemia and hypotension were also recorded in children and adults in a Russian study. Affected Russian children showed slowing of conditioned motor reflexes. Concurrent illness, particularly diarrhea, increases susceptibility to methemoglobinemia. This makes determination of a threshold toxic dose difficult.

People who are particularly sensitive to nitrite develop vasomotor headaches and urticaria.

There is epidemiological evidence suggesting possible human teratogenicity of nitrate polluted drinking water. Human mutations have not been studied in relation to exposure to nitrate or nitrite. Mutagens have been isolated from human gastric contents and identified as nitrosation products of prescription drugs.

Epidemiological studies have correlated high nitrate intake with cancers of the stomach and esophagus. Lower incidences of these cancers correlate with higher intake of fresh fruits and vegetables (particularly those high in vitamin C, which has been shown to inhibit nitrosation in animals). Laboratory studies have related nitrite in foods consumed in areas of high stomach cancer to nitrosamine formation.

VII. MECHANISMS OF TOXICITY

It appears that nitrate toxicity is expressed only after its conversion to nitrite by the action of bacteria. Therefore, the mechanisms of toxicity of nitrate and nitrite are the same. The extent of reaction is dependent on the ion administered, the route of administration, and species differences in distribution of nitrate and metabolic transformation to nitrite. Orally ingested nitrate is absorbed by active transport from the upper part of the small intestine and circulates in the plasma. In humans, about 25% of the nitrate absorbed is eventually secreted in saliva. Oral bacteria reduce an average of 20% of this nitrate to nitrite which is then swallowed (Spiegelhalder et al. 1976). The swallowed nitrite is absorbed by diffusion through the stomach into the blood.

A. Cardiovascular Effect

The acute pharmacological action of nitrite is to relax smooth muscles. The relaxation is nonspecific and affects all smooth muscle irrespective of its innervation or the nature of its responses to adrenergic, cholinergic, or other types of agonists. However, nitrite does not prevent cells from responding to an appropriate stimulus and its effect can be antagonized by any drug that can activate the smooth muscle under consideration. Thus, nitrite is a functional antagonist of norepinephrine, acetylcholine, histamine and many other agents (Nickerson 1975).

The most prominent and important actions of nitrite are on vascular smooth muscle. The cardiovascular response is dominated by changes in venous return and is very sensitive to gravitational influences. Central venous pressure is decreased and may fall to negative values with tilting to the upright position. There may be an initial brief increase in cardiac output during which blood from thoracic reservoirs is shifted to the systemic bed. Subsequently the cardiac output falls. The systemic arterial pressure is usually decreased; the systolic pressure falls more than the diastolic and the latter may be unchanged or decreased only transiently. A small pulse pressure reflects a decreased cardiac stroke volume (Nickerson 1975).

An occasional individual shows marked sensitivity to the hypotensive effects of nitrite. Severe hypotensive responses can occur, particularly when there is little compensatory tachycardia. These responses are associated with nausea and vomiting, weakness, restlessness, pallor, cold sweat and collapse (Nickerson 1975).

B. Effect on Hemoglobin

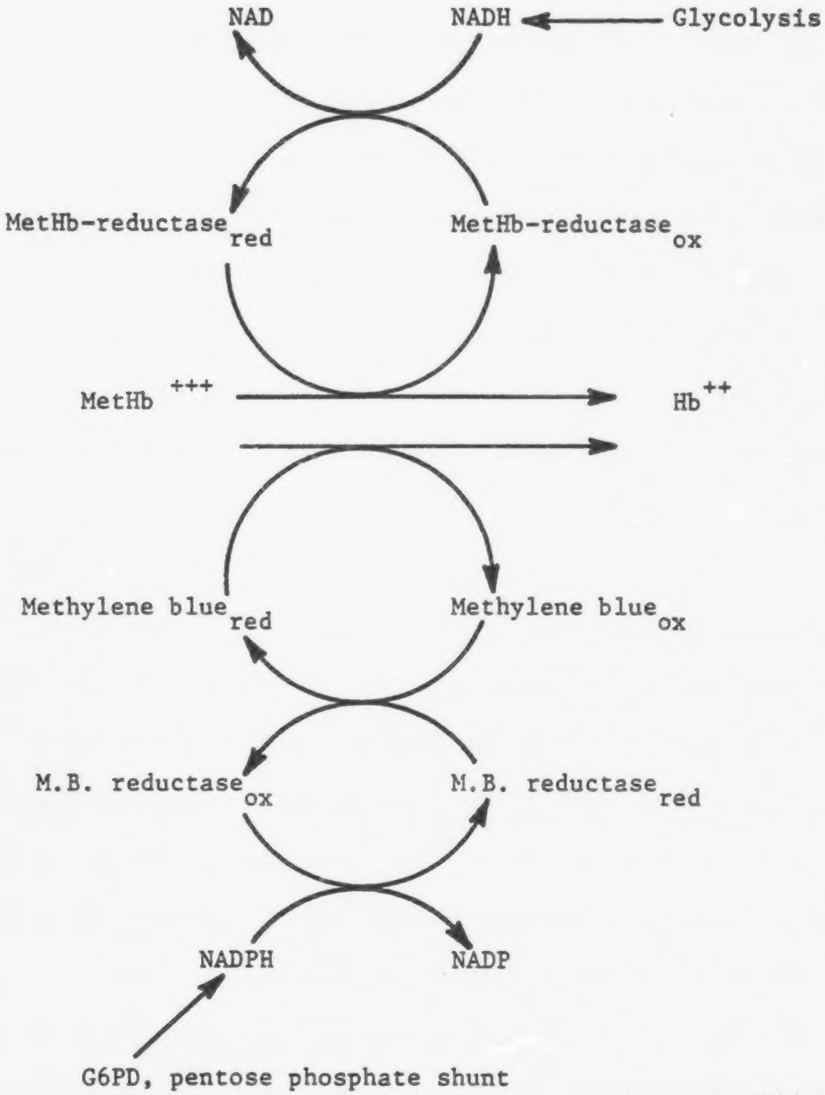
Nitrite reacts with hemoglobin in the erythrocytes, oxidizing the iron of hemoglobin to the ferric state. Ferric hemoglobin is called methemoglobin and is unable to transport oxygen (Parks et al. 1981).

During formation of methemoglobin, superoxide ion (O_2^-) is also formed. The superoxide ion is converted by superoxide dismutase to hydrogen peroxide

(H_2O_2). This in turn forms a catalase-H_2O_2 complex, for which additional nitrite may act as a substrate and be oxidized to nitrate (Parks et al. 1981).

The major system responsible for methemoglobin reduction in mammalian red cells is methemoglobin reductase or diaphorase. As indicated in Figure VII-1, this intracellular enzyme requires NADH as a cofactor. Normal human and most mammalian erythrocytes possess a second reductive system that requires NADPH as a cofactor. In most species, the system appears to be dormant, and it is activated only in the presence of exogenously added electron carriers such as methylene blue. The enzyme reduces methylene blue which in turn nonenzymatically reduces methemoglobin (Burrows 1979). Nitrate is released in the process (Smith and Beutler 1966).

There are large species differences in the rate of reaction of nitrite with hemoglobin to form methemoglobin. Formation is paralleled by similar differences in the rate of reduction by diaphorase to form functional hemoglobin (Smith and Beutler 1966). This is important in determining how sensitive a species is to the vasodilator effects of nitrite and resulting cardiovascular collapse. Species which rapidly remove nitrite from the plasma by reaction with hemoglobin (e.g., ruminants) do not show cardiovascular effects. Species which remove nitrite by this mechanism very slowly (e.g., horses) die of cardiovascular collapse before forming enough methemoglobin to appear cyanotic. Humans fall between these species in removal rate and exhibit both kinds of symptoms concurrently. Different individuals respond with varying sensitivity.


NAD
NADH
Glycolysis
MetHb-reductase$_{red}$
MetHb-reductase$_{ox}$
MetHb^{+++}
Hb^{++}
Methylene blue$_{red}$
Methylene blue$_{ox}$
M.B. reductase$_{ox}$
M.B. reductase$_{red}$
NADPH
NADP
G6PD, pentose phosphate shunt

The spontaneous (NADH) and the dormant (NADPH) methemoglobin reductase systems. Methemoglobin (MetHb) reductase is active in intact red cells in the presence of substrates that can provide for NAD reduction. The NADPH system requires intact red cells, glucose or its metabolic equivalent, a functioning pentose phosphate shunt, and methylene blue (M.B.). M.B. reductase reduces M.B., which in turn nonenzymatically reduces MetHb. Adapted from Smith (1980).

Figure VII-1 Reduction of Methemoglobin

C. Chronic Effects

The diuretic effect of very high doses of nitrate in dogs was attributed to enhancement of sodium excretion. This resulted in a net loss of sodium from the body when nitrate was given as the sodium salt (Greene and Hiatt 1954). The rate of excretion of sodium administered with nitrate was much greater than its rate of excretion when given with chloride. In the dog, as much as 40% more sodium was excreted than was injected (as sodium nitrate), bringing about a loss of extracellular fluid.

Studies showing destruction of the antioxidant vitamins A, beta-carotene and E by nitrite did not define the mechanism. The study authors assumed this involves the oxidation of the vitamins during reduction of nitrite, as is the case with vitamin C (Archer et al. 1975).

Studies on organ pathology, showing dilatation of bronchi and coronary arteries and damage to the liver and spleen, reflect the vasodilation and destruction of hemoglobin discussed previously.

Reduction of motor activity in mice was postulated to be due to formation of metmyoglobin in the muscles, analogous to the formation of methemoglobin in blood (Shuval and Gruener 1977). No mechanism for reactivation of myoglobin was proposed. The same authors proposed no mechanism to explain their observation of abnormal electrical activity in the brains of rats.

D. Effects Due to N-Nitrosation

N-Nitrosation of suitable nitrogenous substrates by nitrite in the acid stomachs of laboratory rodents has been demonstrated by many investigators. Many examples of teratogenesis, mutagenesis and carcinogenesis by this method have been published. Substrates for N-nitrosation by nitrite include secondary amines (which form nitrosamines), N-alkylureas, N-alkylcarbamates and simple N-alkylamides (which form nitrosamides). Tertiary amines can also be nitrosated. Most of these reaction products break down to release nitrosodimethylamine or nitrosodiethylamine (Mirvish 1975).

The amount of nitrosocompound produced in the stomach depends partly on the nitrosation kinetics. For nitrosation to occur, nitrite is first converted to HNO_2 (pK_a3.37). This explains why nitrosation is catalyzed by acid. The reaction rate shows a maximum at pH 3.4. Nitrous acid is then converted to an active nitrosating species, usually nitrous anhydride (N_2O_3) (Mirvish 1975).

Mirvish (1975) concluded the rate of nitrosation is proportional to the concentration of amine and the square of the concentration of nitrite. Therefore, the rate is affected more by an increase in nitrite than by an increase in amine. The rate constants for many secondary amines have been determined. The rate increases as the basicity of the amine decreases (as the pK_a of the amine decreases). Therefore, weakly basic amines react very rapidly.

Thiocyanate increases the rate of nitrosation because the nitrosating species (ON·NCS) is produced. The optimum pH for nitrosation at high concentration of thiocyanate is 2.0. At this point, nitrite becomes fully protonated. Below pH 2.0, the rate falls because NCS^- becomes protonated. Thiocyanate-catalyzed nitrosation of amines will compete favorably with the N_2O_3 mechanism shown above under three conditions: (1) at pH less than 2.5, (2) at high thiocyanate concentration, or (3) at low nitrite concentration (Mirvish 1975).

Mirvish (1975) concluded that the rate of formation of N-nitroso compounds in the stomach is proportional to the thiocyanate concentration as well as the concentrations of amine and nitrite. Thiocyanate is secreted in human saliva along with nitrate and swallowed into the stomach. Boyland and Walker (1974) showed that at pH 1.5, 1.0 mM thiocyanate increases the rate of the nitrosation reaction about 550 times. Human gastric juice is between pH 1 and 2.

Ascorbic acid in the stomach reacts rapidly with nitrite to reduce it and block the nitrosation reaction. Ascorbic acid is oxidized to dehydroascorbic acid in the process. The blocking is most efficient under anaerobic conditions (Archer et al. 1975).

E. Summary

The acute pharmacological action of nitrite is to relax smooth muscle. This effect on vascular smooth muscle can lead to cardiovascular collapse resulting from vasodilation.

Nitrite is also cleared from the blood by its reactions with hemoglobin to form inactive methemoglobin. Methemoglobin is then reactivated with the formation of nitrate which has no vasodilator effect. Conversion of more than 50% of the hemoglobin to the inactive form can be lethal.

Nitrite reacts with various other nitrogenous compounds to form teratogenic, mutagenic and carcinogenic N-nitroso compounds. The reaction occurs in the acid medium of the stomach and can be catalyzed by thiocyanate ion or inhibited by ascorbic acid.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Acceptable Daily Intake (ADI) is calculated. An Adjusted Acceptable Daily Intake (AADI) and Health Advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day)(Body Weight in kg)}}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking Water Volume in L/day}} = \text{mg/L}$$

where:

NOAEL = no-observed-adverse-effect level.

LOAEL = lowest-observed-adverse-effect level.

Body weight = 70 kg for adult or 10 kg for child.

Drinking water volume = 2 L per day for adults or 1 L per day for children.

Uncertainty/Safety Factor = 10, 100 or 1,000.

Utilizing these equations, the following drinking water concentrations are developed for non-carcinogenic effects:

1. A one-day HA for 10-kg child.
2. A one-day HA for 70-kg adult.
3. A ten-day HA for 10-kg child.
4. A ten-day HA for 70-kg adult.
5. A lifetime AADI for a 70-kg adult.

The distinctions made between the HA calculations (items 1 through 4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1 to 2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (Item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon non-carcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS 1977, 1980) is typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.

- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.

- An uncertainty factor of 1,000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data that identify a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines for selection of an uncertainty factor, but must be considered include: (1) the quality of the toxicology data, (2) the significance of the adverse effect and (3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical via drinking water. There is insufficient evidence to classify nitrate as a carcinogen following oral exposure. There is, however, some evidence to indicate that nitrite, in the presence of amines, may be classified as a potential carcinogen.

The presence of amines is a key factor here because other evidence suggests that nitrite in the absence of amines is not carcinogenic. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for non-continuous animal feeding, non-lifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 liters of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as (1) incomplete knowledge concerning the health effects of contaminants in drinking water, (2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results and (3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

A. Non-Carcinogenic Effects

The toxicity of nitrate in humans is due to its reduction to nitrite. This reaction takes place in saliva of humans at all ages and in the gastric fluid of infants during the first three months of life. The toxicity of nitrite takes two forms which vary greatly in severity among animal species: (1) nitrite can cause a vasodilatory effect resulting in cardiovascular collapse and (2) nitrite can also oxidize hemoglobin to methemoglobin resulting in asphyxia. The normal methemoglobin level in humans has been shown to be between one and two percent (Shuval and Gruener 1977). Petukhov and Ivanov (1970) have shown an increase in methemoglobin levels to five percent after drinking water with a high nitrate content (105 mg/L nitrate ion). These facts have led most researchers (see below) to assume that a methemoglobin level of >3% in humans is abnormal. This limit (>3%) is used to define methemoglobinemia and is, therefore, selected as the level which constitutes an adverse effect in humans.

The vasodilatory effect of nitrite can be severe (Weiss et al. 1937, Bartik 1964), but ingesting such a dose from drinking water would require the consumption of two liters of water containing twenty times the current standard for nitrate-nitrogen (10 mg/L) in a matter of a few minutes, coupled with equally prompt conversion of the nitrate to nitrite in the saliva. Recovery from nitrite-induced cardiovascular collapse is more rapid than salivary nitrite production. Therefore, this effect is of primary concern following direct administration of nitrite through pharmaceutical or food additive uses.

The presence of nitrate in water is frequently associated with other contaminants, notably bacteria and nitrites. Microbiological contamination is already regulated, but nitrite has traditionally been omitted from drinking water regulations. It has been assumed that the reducing conditions necessary for the preservation of the nitrite species are uncharacteristic of properly maintained public drinking water supplies and that water sufficiently polluted to foster the presence of nitrites would exceed the microbiological contaminant levels. This dependence on microbiological limits may not be adequate because nitrites are detected in water which does meet the U.S. Environmental Protection Agency coliform limits.

1. Short-Term Exposure

Shuval and Gruener (1977) treated groups of 12 pregnant "sabra" rats through weaning with 2,000 or 3,000 mg/L nitrite ion (NO_2^-) as sodium nitrite in drinking water. This represents a daily dose of 267 or 400 mg nitrite/kg body weight. Seven controls were administered tap water. During the period from birth to weaning, the newborns showed no abnormally high methemoglobin. These results are in contrast to the report by Donahoe (1949) that a human infant suffered clinical methemoglobinemia while breast-feeding from a mother who drank water from a well suspected of nitrate contamination. Blood of near-term rat fetuses has approximately 10 times the methemoglobin reductase activity of human cord blood (Shuval and Gruener 1977). Thus, it appears that the newborn rat is very resistant to methemoglobinemia and is not a suitable model for nitrate and nitrite toxicity in the human infant.

Shuval and Gruener (1977) treated 50-day-old male C_{57}bl/6J mice (15 per group) with 0, 100, 1,000, 1,500 or 2,000 mg nitrite ion (as sodium nitrite) per liter of drinking water (reported to be 0, 8.8, 88, 133 or 178 mg nitrite ion/kg/day) for three weeks. Motor activity and methemoglobin levels were studied. No change was observed at 8.8 mg/kg/day (metHb = 0.67% versus 0.59% in controls) and the changes in motor activity and methemoglobin levels at 88 mg/kg/day (metHb = 1.27%) were reported to be insignificant. A dose of 133 mg/kg/day increased methemoglobin levels to 2.71% (2% is the $P=0.05$ level in mice for this study) while a dose of 178 mg nitrite ion/kg/day produced a marked increase in methemoglobin levels (to 10.14%) and a significant decrease in motor activity in the mice. Therefore, 88 mg nitrite ion/kg/day was determined to be the NOAEL in this study.

2. Long-Term Exposure

Several human studies demonstrating elevated levels of methemoglobin resulting from consumption of water containing elevated nitrate levels have been reported. These studies have been presented in detail in Section VI and are summarized here in Table VIII-1. The studies by Super et al. (1981) in Southwest Africa and by Petukhov and Ivanov (1970) in the Soviet Union suggest that older infants in these countries retain a high sensitivity to nitrate toxicity beyond three months of age. Generally, infants in the Western countries exhibit a reduced sensitivity after three months. Since this difference is likely to be cultural (i.e., difference in diet, vitamin C, etc.) (Super et al. 1981), it would be inappropriate to use these studies for calculating HAs for children in the U.S.

Donahoe (1949) reported five cases of methemoglobinemia in infants less than three-months-old. All cases could be traced to well water contaminated with nitrate. One case involved a newborn breast-fed infant whose mother drank nitrate-contaminated well water. In another case an infant was fed milk from cows drinking nitrate-contaminated water. This strongly indicates that nitrate is excreted into milk of humans and cows. Cyanosis appeared within a few days to two weeks after feeding formula made with the contaminated water, mother's milk or cow's milk. In two cases the nitrate concentration of the contaminated water was measured. The levels found were 50 and 117 mg/L nitrate, which is equal to 11.3 and 40 mg/L nitrate-nitrogen.

Walton (1951) published a survey by the American Public Health Association which found over 278 cases of cyanosis in infants definitely associated with nitrate-contaminated water. No cases associated with water containing 10 mg/L or less of nitrate-nitrogen were found.

Table VIII-1 Health Effects of Nitrate-Nitrogen in Humans

Author	Age	Effect	Nitrate-Nitrogen NOAEL	Nitrate-Nitrogen LOAEL
Donahoe 1949, USA	0-2 months	cyanosis		11.3 mg/L
Walton 1951, USA	infants	cyanosis	10 mg/L	11-20 mg/L
Simon et al. 1964, Germany	0-3 months	cyanosis, diarrhea		<4.6 mg/L
	0-3 months	cyanosis, diarrhea(?)		<11.3 mg/L
Toussaint & Wurkert 1982, Germany	young infants	mean metHb >3%	11.3 mg/L	11.5-23 mg/L
Winton et al. 1971, USA	0-6 months	metHb ≥3%	14.1 mg/L	
Shearer et al. 1972, USA	0-3 months	metHb ≥4%		<6.7 mg/L
	3-6 months	metHb ≥4%	27 mg/L	
Gruener & Toeplitz 1975, Israel	0-10 months	metHb ≥6%		25 mg/L
Super et al. 1981, S.W. Africa	1-2 months	metHb ≥3%		<20 mg/L
	3-4 months	metHb ≥3%		<20 mg/L
	5-6 months	metHb ≥3%		<20 mg/L
	7-8 months	metHb ≥3%		<20 mg/L
	9-10 months	metHb ≥3%		<20 mg/L
	11-12 months	metHb ≥3%		<20 mg/L
Craun et al. 1981, USA	1-8 years	mean metHb >3%	111 mg/L	
Petukhov et al. 1972, USSR	6 months to adult	cyanosis		24 mg/L

Simon et al. (1964) reported results of a survey of 745 cases of infant methemoglobinemia. Nitrate concentrations in the drinking water were less than 100 mg/L in 16.2% of the cases, and less than 50 mg/L in 4.4% of cases. Of the infants surveyed, 97.7% were less than three-months-old and 53% had diarrhea. The authors suggested that cases of infant methemoglobinemia associated with low-nitrate water might be due to feeding spinach with high nitrate content but there was no identification of which cases were associated with diarrhea. Since 11.8% of the cases were using drinking water with nitrate between 50 and 100 mg/L, a conservative interpretation would be to use 50 mg/L nitrate (11.3 mg/L nitrate-nitrogen) as the LOAEL for this study. However, the high incidence of diarrhea makes this study of questionable value for standard setting.

Winton et al. (1971) compared methemoglobin levels with nitrate ion intake in 111 infants less than six-months-old. Only three infants had methemoglobin levels above 2.9%. They were the youngest of five infants who had received more than 10 mg/kg/day of nitrate ion. The NOAEL for this study is 10 mg/kg/day of nitrate ion, or 14.1 mg/L nitrate-nitrogen for a 4-kg infant.

Toussaint and Wurkert (1982) studied the methemoglobin levels of 68 infants in Germany as a function of the nitrate concentration in drinking water. They found mean methemoglobin levels below 3% at nitrate concentrations below 50 mg/L, or 11.3 mg/L nitrate-nitrogen, in healthy infants.

Craun et al. (1981) conducted an epidemiologic study of 102 children aged one to eight years in Washington County, Illinois. Of the study subjects, 64 were consuming high nitrate water (22 to 111 mg/L nitrate-nitrogen) and 38 were consuming low nitrate water (less than 10 mg/L nitrate-nitrogen). Ingestion of water containing 22 to 111 mg/L nitrate-nitrogen did not produce abnormal mean methemoglobin levels and was not related to increased methemoglobin levels in comparison to controls. In the entire study group of 102 children, only five had methemoglobin levels greater than 2% (maximum of 3.1% in a child from the low exposure group).

It should be noted that drinking water is not the only source of nitrate that can cause infant methemoglobinemia. Holscher and Natzschka (1974) reported two cases of infants less than four-months-old who developed cyanosis from methemoglobinemia after being fed fresh spinach which contained high levels of nitrate ion. Simon et al. (1964) doubted the significance of the finding of a few cases of methemoglobinemia in infants administered water containing less than 20 mg/L nitrate (4.6 mg/L nitrate-nitrogen). In this study diarrhea was common. Subsequently, Hegesh and Shiloah (1982) demonstrated that acute diarrhea in infants results in endogenous, _de novo_ synthesis of nitrites causing methemoglobinemia. Infections including diarrhea were also implicated in elevated methemoglobin levels in the studies of Shearer et al. (1972) and Toussaint and Wurkert (1982). Studies of infants without reported concurrent infection will be used for determining HA values, but nitrate exposures which are safe for healthy infants may contribute to methemoglobinemia in infants suffering from diarrhea or other infections.

Newberne (1978, 1979) fed sodium nitrite to groups of 68 Sprague-Dawley rats of each sex in various diets (Table VIII-2) from the time of conception through their natural life span. Most groups had 50% survival after 100 weeks (only the positive control groups did not). The usual spectrum of neoplastic and non-neoplastic diseases of older rats was observed. Histopathological lesions related to nitrite exposure were observed in the spleen, lymph nodes and other components of the lymphoreticular tissues and were diagnosed by the authors as either malignant lymphoma or immunoblastic cell proliferation. Lesions identified as malignant lymphomas were significantly increased in all nitrite-treated groups (the combined incidence of lymphomas in groups that were not fed nitrite was 5.4% compared to 10.2% in the combined nitrite-treated groups) (Newberne 1979). Statistical analysis of nonmalignant lesions was not reported.

Because of differences of opinion over the diagnoses of malignant lymphoma or immunoblastic cell proliferation, a Joint Committee of Experts was asked to review all of the histological slides from the Newberne study (FDA 1980a). The committee members diagnosed many of the reported lymphomas as nonmalignant lesions including extramedullary hematopoiesis, plasmacytosis or histiocytic sarcoma. The committee interpreted the lesions which Newberne called immunoblastic cell proliferation as extramedullary hematopoiesis, plasmacytosis or reactive lymphoid hyperplasia.

Table VIII-2 Carcinogenicity Study Results

Treatment	Group	Nitrite ion (NO_2^-) mg/kg/day[a]	Proportion of rats with: Malignant lymphomas	Proportion of rats with: Immunoblastic cell proliferation
In semipurified agar diet[b]	1	0	5/136	10/136
	2	12.5	10/136	9/136
	3	25	11/136	23/136
	4	50	11/136	14/136
	5	100	14/136	23/136
0.1% in water (agar diet)	6	100[c]	16/136	17/136
0.2% in water (agar diet)	7	200[c]	14/136	18/134
Positive control 2,000 ppm urethane (agar diet)	8		37/135	0/135
Commercial chow diet (Purina)	9	0	9/132	5/132
	10	50	14/134	12/134
	11	100	12/132	11/132
Positive control 2,000 ppm urethane (chow diet)	12		19/136	0/136
Casein diet	13	0	10/136	10/136
	14	50	18/136	11/136
Agar diet (mothers of groups 1 and 4)	15	0	1/33	8/33
	16	50	6/34	8/34
Nitrite exposure after weaning (agar diet)	17	0	6/136	21/136
	18	50	6/131	18/131

[a] Unless otherwise noted.

[b] Ingredients of Agar Diet:

Casein	220
Sucrose	147
Dextrose	197
Dextrin	197
Corn Oil (Mazola)	150
Vitamin Mix	10
Mineral Mix	40
Agar	35
Distilled Water	1,000

[c] Calculated by assuming water consumption of 10% of body weight per day.

Adapted from Newberne 1978, 1979.

Regardless of whether or not the histologic lesions noted above were malignant or precursors of malignancy, the Newberne study demonstrated that the incidence of lesions was related to nitrite intake. Table VIII-2 shows that for groups of 136 rats fed diets containing 0, 12.5, 25, 50 or 100 mg/kg/day nitrite ion (as sodium nitrite), rats with lesions of the lymphoreticular system totaled 15, 19, 34, 25 and 37, respectively (Newberne 1979). The NOAEL appears to be 12.5 mg/kg/day nitrite ion or 3.8 mg/kg/day nitrite-nitrogen.

Petukhov and Ivanov (1970) provided evidence that long-term exposure to high nitrate levels in drinking water may directly affect the central nervous system in humans. The authors described the slowing of conditioned motor reflexes in response to auditory and visual stimuli in 39 Russian children whose drinking water contained nitrate at 105 mg/L. The reflexes of these children were compared with those of a group of 20 children whose drinking water contained 8 mg/L. Children drinking the high nitrate water had average methemoglobin levels of 5.3% while the controls averaged 0.75%.

B. Quantification of Non-Carcinogenic Effects

The available data suggest that calculation of the HA values and the AADI for nitrate/nitrite should:

- Recognize the newborn infant as the population group at greatest risk.

- Recognize and consider the conversion of orally ingested nitrate to nitrite.

- Utilize human data wherever possible due to the extreme species variation (both quantitative and qualative) observed in nitrate and nitrite toxicity.

Recognition of newborn infants (e.g., those up to three-months-old and approximately 4 to 6 kg) as a special risk group is due to their greater sensitivity to nitrate following oral ingestion. This sensitivity of infants (due to hemoglobin inactivation by nitrite transformed from ingested nitrate) is greater than that of the adult for the following reasons:

- An infant drinks about 160 mL/kg/day of formula (640 mL/4-kg infant) (Davidson et al. 1975), while an adult drinks about 29 mL/kg/day of water (2,000 mL/70-kg adult). This is over five times more water per kg of body weight for an infant.

- The infant's gastric pH is high enough (≥ 4) to permit the growth of nitrate-reducing bacteria (Franklin and Skoryna 1971). Thus, 100% of ingested nitrate is assumed to be transformed to nitrite in the infant. This compares to the assumed maximum of 10% of ingested nitrate transformed by oral microorganisms to nitrite in the saliva of older children and adults.

- The infant's hemoglobin is in a form which is readily affected by nitrite (Phillips 1971).

- The infant's ability to restore hemoglobin enzymatically to its functional state is low (Gruener et al. 1973, Ross and Desforges 1959).

Thus, HA values are presented below for a 4-kg infant (assumed to consume 0.64 L of formula per day), as well as a 70-kg adult. Normally, HAs are determined for the 10-kg child and the 70-kg adult. However, in this case, the newborn infant (assumed to weigh 4-kg) is the population at greatest risk and HAs are provided for the 4-kg infant. While no separate HAs for the 10-kg child are provided, the HAs for the 70-kg adult will be protective of all age groups, other than the 4-kg infant, in that they are based on data obtained in children (Craun et al. 1981).

For the HAs and AADI, nitrate and nitrite cannot be considered independently. This is because the toxic effects of nitrite can be caused by the ingestion of nitrites and/or the ingestion of nitrates with subsequent transformation to nitrite. Gastrointestinal absorption factors for nitrate and nitrite and the percent transformation of nitrate to nitrite greatly influence this interrelationship. The rate of gastrointestinal absorption and/or nitrate to nitrite transformation is a function of several factors including stomach pH and compounds present in the stomach (Friedman et al. 1972). Nitrate which is not transformed into nitrite passes into the small intestine where it is absorbed at an unknown rate (Witter et al. 1979b).

It is generally assumed, however, that nitrate absorption in the small intestines is rapid and approaches 100%. Nitrite ions are rapidly absorbed (85% to 100% in less than ten minutes) through the gastric mucosa (Parks et al. 1981). The following factors are assumed:

- The percent of ingested nitrate that is absorbed through the small intestines is 100% for the 70-kg adult.
- The percent of ingested nitrate that is absorbed and transformed to nitrite in the saliva is 10% for the 70-kg adult (Friedman et al. 1972).
- The percent of ingested nitrate that is transformed to nitrite in the stomach is 100% for the 4-kg infant.
- The percent of ingested or transformed nitrite that is absorbed through the gastric mucosa is 100% in all cases (Parks et al. 1981).

Each HA and the AADI is presented in terms of both mg/L nitrate-nitrogen and mg/L nitrite-nitrogen in drinking water. Appropriate use of these values requires information on both the nitrate and nitrite content of the drinking water so that a total "effective" concentration for nitrate can be calculated and used as follows:

- The "effective" nitrate-nitrogen concentration (mg/L) = nitrate-nitrogen concentration (mg/L) + (10.0) nitrite-nitrogen concentration (mg/L).

- The "effective" nitrate-nitrogen concentration (mg/L) should not exceed the appropriate nitrate-nitrogen value for the appropriate age group (4-kg infant or 70-kg adult) or exposure period.

1. One-Day Health Advisory

No suitable studies for derivation of a one-day HA were located. The ten-day HAs should be protective of one-day exposures.

2. Ten-Day Health Advisory

a. Seventy Kilogram Adult

The study by Craun et al. (1981) is selected as the basis for the ten-day HA for the 70-kg adult. The authors evaluated mean methemoglobin levels in 64 children aged one- to eight-years-old who were exposed to 22 to 111 mg/L nitrate-nitrogen in their drinking water, and in thirty-eight controls who were exposed to <10 mg/L nitrate-nitrogen in their drinking water (total of 102 subjects). No cyanosis or elevation of mean methemoglobin were observed at these levels. Since higher concentrations were not evaluated, this may not represent the maximum no effect level. Because the children varied considerably in age and size, a mg/kg/day equivalent dose is not estimated. This, however, is not necessary since the reverse calculation to mg/L of drinking water would be required in developing an HA. Also, since the study involved the oral exposure to nitrate-nitrogen in drinking water, no assumptions regarding absorption and transformation of nitrate to nitrite are required.

Nitrate-Nitrogen Ten-Day Health Advisory for 70-kg Adult:

The ten-day HA for a 70-kg adult (and all age groups other than the infant) is 111 mg/L nitrate-nitrogen. This value is derived by direct use of the NOAEL of 111 mg/L observed by Craun et al. (1981), based on no cyanosis or elevation in mean methemoglobin levels in one- to eight-year-old children exposed to nitrate in water. Since the study was based on observations in humans, and since the most sensitive subgroup of the population (i.e., infants) is considered separately (below), no uncertainty factor has been employed in deriving the HA from the NOAEL.

Nitrite-Nitrogen Ten-Day Health Advisory for 70-kg Adult:

There are no studies which provide a direct measure of the NOAEL for nitrite in noninfants. The ten-day nitrite HA for a 70-kg adult (and all age groups other than the infant) can be calculated from the NOAEL for nitrate, assuming 10% conversion of nitrate to nitrite, as follows:

$$111 \text{ mg/L nitrate-nitrogen } (0.1) = 11 \text{ mg/L nitrite-nitrogen}$$

where:

111 mg/L = NOAEL for nitrate in children, based on no cyanosis or elevation in mean methemoglobin levels (Craun et al. 1981).

0.1 = Assumed 10% conversion of nitrate to nitrite by microorganisms in the saliva of noninfants.

b. Four-Kilogram Infant

The study by Walton (1951) is selected as the basis for the ten-day HA for the 4-kg infant. This survey of nearly 300 cases of methemoglobinemia in infants in the United States found no cases associated with water containing less than 10 mg/L of nitrate-nitrogen. Even though this study involves chronic exposure, it was selected in preference to the other studies of infants presented in Table VIII-1 because it determined the lowest NOAEL, it surveyed a large population, it was not complicated by diarrhea as were the data of Simon et al. (1964) and Shearer (1972), it did not require conversion of the dose from mg/kg/day to mg/L which could introduce errors as in Winton et al. (1971) and it was a survey of methemoglobinemia in the United States unlike Simon et al. (1964) and Toussaint and Wurkert (1982).

Nitrate-Nitrogen Ten-Day Health Advisory for 4-kg Infant:

The 10-day HA for infants is 10 mg/L nitrate-nitrogen. This HA is derived by direct use of the NOAEL of 10 mg/L for methemoglobinemia in a survey of nearly 300 infants (Walton 1951). Since the study considers the most sensitive subgroup of the human population (i.e., infants), no uncertainty factor has been employed in deriving the HA from the NOAEL.

It should be noted from Table VIII-1 that this HA for nitrate-nitrogen for a 4-kg infant is supported by the studies by Winton et al. (1971), Toussaint and Wurkert (1982) and Donahoe (1949).

Nitrite-Nitrogen Ten-Day Health Advisory for 4-kg Infant:

No study provides a direct measure of the NOAEL for nitrite in infants. However, the ten-day nitrite HA for the 4-kg infant can be calculated from the NOAEL for nitrate as follows:

$$\frac{10 \text{ mg/L nitrate-nitrogen}}{10} = 1 \text{ mg/L nitrite nitrogen}$$

where:

10 mg/L = NOAEL for nitrate-nitrogen, based on absence of methemoglobinemia in infants exposed to nitrate in drinking water (Walton 1951).

10 = Uncertainty factor. This factor has been selected because the NOAEL employed is for nitrate while nitrite is the toxic species, and there is uncertainty regarding the rate and extent of nitrate transformation to nitrite.

3. Adjusted Acceptable Daily Intake

As discussed earlier, the AADI for nitrate and nitrite should be at a level which protects the most sensitive subgroup of the human population (i.e., infants). Therefore, no AADI values are derived for children or adults. The study by Walton (1951) on reported cases of methemoglobinemia in infants (diagnosed by observation of cyanosis) has been selected to serve as the basis for the AADI. This survey of nearly 300 cases of methmeoglobinemia in infants in the United States found no cases associated with water containing less than 10 mg/L of nitrate-nitrogen. This study was selected in preference to other studies of infants (see Table VIII-1) because it surveyed a large population and it was not complicated by associated cases of diarrhea.

Nitrate-Nitrogen AADI:

The AADI for nitrate-nitrogen is 10 mg/L. This value is derived by direct use of the NOAEL of 10 mg/L nitrate-nitrogen observed by Walton (1951) in a study of methemoglobinemia in over 300 human infants exposed to nitrate in drinking water. Since the study involved the most sensitive subgroup of the human population (i.e., infants), no uncertainty factor has been employed in deriving the AADI from the NOAEL.

Nitrite-Nitrogen ADDI:

No study was found which provides a direct estimate of the NOAEL for nitrite in human infants. However, the NOAEL for nitrate in infants identified by Walton (1951) may be used to estimate an AADI for nitrite, as follows:

$$\frac{10 \text{ mg/L nitrate-nitrogen}}{10} = 1 \text{ mg/L nitrite nitrogen}$$

where:

10 mg/L = NOAEL for nitrate-nitrogen reported for methemoglobinemia in human infants (Walton 1951).

10 = Uncertainty factor. This factor has been selected because the NOAEL employed is for nitrate while nitrite is the toxic species, and there is uncertainty regarding the rate and extent of nitrate transformation to nitrite.

Since these calculations are based upon human exposure data, no calculation is necessary to reflect relative source contribution data.

The U.S. Public Health Service revised its Drinking Water Standard for nitrate in 1962. A recommended limit of 10 mg/L nitrate-nitrogen or 45 mg/L nitrate ion was determined (USPHS 1962). This was based on the survey of case reports of methemoglobinemia and the absence of cases when the water contained less than 10 mg/L nitrate-nitrogen by Walton (1951).

At the request of the Environmental Protection Agency, the Committee on Water Quality Criteria of the National Academy of Sciences published Water Quality Criteria in 1972, including a recommendation as follows:

> "On the basis of adverse physiological effects on infants and because the defined treatment process has no effect on the removal of nitrate, it is recommended that the nitrate-nitrogen concentration in public water supply sources not exceed 10 mg/L. On the basis of its high toxicity and more pronounced effect than nitrate, it is recommended that the nitrite-nitrogen concentration in public water supply sources not exceed 1 mg/L." (NAS 1972 p. 73.)

The Environmental Protection Agency published Quality Criteria for Water in 1976 and reached this conclusion:

> "Because of the potential risk of methemoglobinemia to bottle-fed infants, and in view of the absence of substantiated physiological effects at nitrate concentrations below 10 mg/L nitrate-nitrogen, this level is the criterion for domestic water supplies. Waters with nitrite-nitrogen concentrations over 1 mg/L should not be used for infant feeding. Waters with a significant nitrite concentration usually would be heavily polluted and probably bacteriologically unacceptable." (USEPA 1976a p. 203).

The current Maximum Contaminant Level (MCL) for nitrate-nitrogen remains 10 mg/L (40 CFR 141, USEPA 1976b). This MCL is also based on case reports of methemoglobinemia diagnosed by observation of cyanosis (Walton 1951).

C. Carcinogenic Effects

Many studies have documented carcinogenesis (adult and prenatal) in animals administered nitrite orally with nitrosatable compounds (NAS 1981). Tumors were induced in many organs including the stomach, esophagus and nasal cavity. Carcinogenesis in several of these experiments could be inhibited by simultaneous oral administration of ascorbic acid. In one study (Mirvish et al. 1976), liver tumors were decreased by ascorbate, but forestomach tumors developed instead.

Over 120 N-nitroso compounds have been tested for carcinogenicity. Greater than 75% have been shown to be carcinogenic (Shank and Magee 1981). These compounds have been demonstrated to be carcinogenic in at least 22 species and carcinogenic transplacentally in at least five species (Schmahl and Habs 1980). All species tested have shown tumor formation following treatment with one or more N-nitroso compound. Further, tumors have been induced in every organ and tissue and from most types of cells. The responses show organ specificity within a species, even after administration by different routes. However, striking differences in target tissue are seen between species (Lijinsky et al. 1975).

A number of inadequate older animal studies provide inconclusive evidence regarding the carcinogenicity of nitrate and nitrite administered in the absence of nitrosatable compounds. The study by Newberne (1978, 1979) is inconclusive due to conflicting opinions of the pathologists that examined the slides. The most recent carcinogenic bioassay of nitrate and nitrite (Maekawa et al. 1982) used appropriate doses and numbers of rats, but the strain used, F-344, had a nearly 100% incidence of tumors in the control animals. In spite of this limitation, it was concluded that neither nitrate nor nitrite ingestion caused significant differences in mean time to tumor, increase of malignant tumors or increase of any specific tumor.

Several human epidemiological and case studies are suggestive of a relationship between nitrate and/or nitrite ingestion and cancers of the stomach and esophagus. Lower incidences of these cancers correlate with higher intake of fresh fruits and vegetables, particularly those high in absorbic acid which has been shown to inhibit nitrosation in animals. Laboratory studies have related nitrite in foods used in areas of high stomach cancer to nitrosamine formation (NAS 1981). Environmental cancer epidemiology cannot be used to quantify risk from nitrite due to the many confounding factors involved such as the wide variation in exposure to other chemicals and the difficulty of estimating exposure levels.

D. Quantification of Carcinogenic Effects

No studies have provided conclusive evidence to indicate that nitrate or nitrite are carcinogenic. Thus, no quantification of carcinogenic risk has been performed on these chemicals. The International Agency for Research on Cancer (IARC) has not evaluated the carcinogenic potential of nitrate or nitrite (WHO 1982).

Although nitrate and nitrite alone have not been shown to be carcinogenic, there is a potential carcinogenic risk associated with nitrate intake. The potential risk of carcinogenesis relating to nitrate intake appears to be due to its reduction to nitrite in saliva and subsequent reactions of the nitrite with stomach contents to form N-nitroso compounds. The IARC has not evaluated the carcinogenic potential of N-nitroso compounds (WHO 1982); however, there is considerable scientific evidence to support this potential risk.

The Panel on Nitrates of the Coordinating Committee for Scientific and Technical Assessments of Environmental Pollutants, National Academy of Sciences, calculated the risk of human liver cancer from nitrosamines using both high and low estimates of exposure (NAS 1978). To calculate the maximum normal exposure, the Panel assumed: (1) an average daily intake of 100 mg nitrate, (2) an average daily intake of 2 mg nitrite, (3) the salivary conversion of 10% of the nitrate to nitrite, and (4) reaction of 5% of the nitrite to form nitrosodimethylamine. This is equivalent to 481 ppm of nitrosodimethylamine in the diet (food only). To calculate the minimum exposure, concentrations of nitrosamines in the blood of a man before and after eating a meal were measured and extrapolated to the equivalent of 2.2 ppm of nitrosodimethylamine in the diet. This method fails to account for the rapid removal of the nitrosamine from the blood by the liver or other tissues. The level measured at any one time is not the total produced; therefore the estimate is a minimum.

The liver cancer risk from nitrosodimethylamine was then extrapolated from the data of Terracini et al. (1967) on four to six-week-old female rats (Porton strain). Nitrosodimethylamine was administered to the animals at 0, 2, 5, 10, 20 and 50 ppm in their diets for life (50 ppm = 2.5 mg/kg/day). The panel used a modification of the model of Guess and Crump (1976). This calculation gave an excess lifetime risk of liver cancer between a minimum of 1×10^{8} and a maximum of 6×10^{4} resulting from normal intake of nitrates and nitrites in the diet. This assumes that the pharmacokinetics and pharmacodynamics that apply to nitrosamines in the rat also apply in the human.

The current incidence of liver cancer in the United States is about two cases per 100,000 people per year, or about 140 cases in 100,000 70-year lifetimes. Therefore, the calculated risks from estimated exposure to nitrosamines could be expressed as the equivalent of 0.0007% to 43% of the current rate of liver cancer. This risk curve rises very steeply in relation to dose. A 218-fold increase in dose gives 60,000-fold increase in risk at the doses studied. This suggests an exponential dose-response.

The Committee on Nitrite and Alternative Curing Agents in Food, of the Assembly of Life Sciences, National Academy of Sciences, completed an assessment of the carcinogenic impact of all exposure to nitrosamines (NAS 1981). They used different methods, but based extrapolations on the same study by Terracini et al. (1967) used by the panel above. The Committee combined the exposure data from Terracini et al. (1967) with data on exposure to nitrosamines in cosmetics, car interiors, beer, bacon, tobacco smoke and work environments to project total exposures for people having seven different lifestyles (Table VIII-3). Their average lifetime risk prediction due to nitrosamines from all sources is 5.6×10^{-5}, which falls between the high and low estimates for dietary exposure above. Their estimated lifetime increased risk due to drinking water (1.6 L/day) with 100 mg/L nitrate is 2.7×10^{-4}. This is about five times the average risk.

The Committee chose the conservative approach of extrapolating a linear response below $P_o = 0.02$. Thus, their estimates are maximum risks. They chose surface area as the basis for their interspecies extrapolation rather than weight. They made the same assumptions about human sensitivity to carcinogenesis by nitrosodimethylamine as the panel did (NAS 1978). The committee emphasized the great uncertainty of the assumptions used and asserted that the absolute numbers stated are not intended as a guide for policy formation (NAS 1981).

E. Existing Guidelines and Standards

The World Health Organization in a draft document entitled, "Guidelines for Drinking Water Quality-Recommendations (Volume 1)," recommended a limit of 10 mg/L nitrate and less than 1 mg/L nitrite in drinking water (WHO 1983).

Table VIII-3 Estimates of Lifetime Risk of Cancer
Due to Nitrosamine Exposure

Source of Exposure	Nitrosamine Exposure, mg/kg/day	
	Average diet, nonsmoker	Nitrate-rich water,[a] no beer, nonsmoker
Endogenous		
Diet (Total)	0.00002	0.00022
Exogenous		
Cosmetics	0.0000063	0.0000063
Car interiors	0.000003	0.000003
Beer	0.000015	0
Bacon	0.0000026	0.0000026
Tobacco smoke	0	0
Work	0	0
Total exposure	0.000047	0.000232
Lifetime risk	5.6×10^{-5}	2.7×10^{-4}
Range of lifetime risk	8.2×10^{-6} to 1.8×10^{-4}	3.9×10^{-5} to 8.7×10^{-4}

[a] Assuming that consumption of nitrate-rich water provides 2.5 mg/kg/day of nitrate

Adapted from NAS 1981

The World Health Organization in 1978 published the report of the WHO task group on environmental health criteria for nitrates, nitrites and N-nitroso compounds, which concluded:

> "Nitrate levels in public drinking water should comply with, or preferably be lower than, the tentative limit of 45 mg/litre recommended in the International Standards for Drinking Water (WHO 1971). With respect to the carcinogenic risk from exposure to N-nitroso compounds, it is prudent to assume that any exposure may involve some degree of risk, and that exposure should therefore be kept as low as practically achievable." (WHO 1978 p. 84).

F. Special Considerations

1. High Risk Populations

Populations living in areas with high nitrate concentrations in ground water or in soil used for growing vegetables are at increased risk for nitrate/nitrite toxicity, as are populations eating diets high in cured meats.

Populations chronically exposed to industrial air pollution containing nitrosatable amines are at increased risk for carcinogenic N-nitroso compound formation from reaction with nitrite in the stomach (as well as with nitrogen oxides in the air) assuming contamination of hands or of garden produce. A similar situation exists for populations in areas sprayed with any of the pesticides which nitrosate to carcinogenic or teratogenic compounds (Quarles et al. 1979, Teramoto et al. 1980). Many nitrosatable pesticides, including compounds such as carbaryl (Quarles et al. 1979), are allowed to be present as residues on food. Therefore, a very large segment of the population is currently at risk for the adverse effects due to exposure to these nitrosation products.

2. Sensitive Individuals

Persons with unusually low blood pressure or an inability to compensate for lowering of the blood pressure are at increased risk of adverse effects due to vasodilation. This could include persons being treated with diuretics and other drugs which lower blood pressure.

Infants are at greatest risk of developing methemoglobinemia from nitrate exposure. This increased susceptibility is related to at least four factors (Phillips 1971). First, fetal hemoglobin is more readily oxidized than hemoglobin in adults. At birth, between 60% and 80% of the hemoglobin remains in the immature form of the molecule, hemoglobin F. This decreases to approximately 30% by the third month of life (Pisciotta et al. 1959). Second, the infant has a transitory physiologic deficiency of methemoglobin reductase or its cofactor, NADH (reduced nicotinamide adenine dinucleotide), which are necessary to maintain iron in its reduced state (Ross and Desforges 1959). Third, on a weight basis, infants consume over five times as much water (the most important source of nitrate in the etiology of methemoglobinemia) as adults (Burden 1961). Finally, relative achlorhydria in the very young favors overgrowth of nitrate-reducing bacteria in the stomach (Walton 1951).

The sensitivity of hemoglobin to oxidation by nitrite is increased in children, pregnant women and patients with cancer (Metcalf 1961). The increased sensitivity is present in children of both sexes up to the age of puberty. Blood samples from 100 pregnant women, 51 cancer patients, and 100 medical and physiotherapy students were tested. Increased sensitivity of the hemoglobin to oxidation was exhibited in 96% of women more than six weeks pregnant. A similar but less marked change occurred in the patients with cancer. No cases of increased sensitivity occurred in any of the students. In pregnant women, the sensitivity of the hemoglobin to oxidation rapidly returned to normal after delivery. In the few cancer patients examined, the sensitivity of hemoglobin returned to normal following complete surgical removal of the malignant tumor.

Individuals with altered physiological states or with either hereditary or acquired disease may also be predisposed to the development of nitrite- or nitrate-induced methemoglobinemia. These include individuals with glucose-6-phosphate dehydrogenase deficiency, adults with reduced gastric acidity (including those being treated for peptic ulcer or individuals with chronic gastritis or pernicious anemia) and individuals with a rare, hereditary lack of NADH or methemoglobin reductase activity in their red blood cells (Scott 1960). This hereditary enzyme deficiency seems also to account for the somewhat increased incidence of methemoglobinemia among Alaskan Eskimos and Indians (Scott and Hoskins 1958). Some Caucasian ethnic groups, including Sardinians, Sephardic Jews, Greeks and Iranians have an erythrocyte glucose-6-phosphate dehydrogenase deficiency. A similar deficiency has been demonstrated in approximately 13% of black males in Illinois (Tarlov et al. 1962). These individuals are less able to reduce methemoglobin to hemoglobin in response to the nitrite antidote, methylene blue (see Figure VII-1).

3. Synergistic Effects

Individuals at greater than normal risk for formation of hazardous N-nitroso compounds include those taking nitrosatable prescription drugs such as cimetidine (Tagamet[a]) in the treatment of stomach ulcers, the tranquilizer diazepam (Valium[a]) (Stemmermann et al. 1981), the anthelmintic piperazine (Greenblatt and Mirvish 1972), disulfiram (Antabuse[a]) in the treatment of alcoholism, methapyrilene (Dormin[a]) for relief of insomnia (Lijinsky 1974) and many others.

The risk of formation of N-nitroso compounds in the stomach may be greatly enhanced in smokers due to the production of the nitrosation catalyst thiocyanate ion. Cigarette smoke contains cyanide compounds which are converted to thiocyanate in the body. The saliva of smokers contains three to four times as much thiocyanate as that of nonsmokers and this could contribute to the excess cancer risk of smokers (Boyland and Walker 1974).

4. Antagonistic Effects

Laboratory and epidemiological studies support the value of vitamin C in preventing the nitrosation of various substrates by nitrite, therefore, reducing the cancer risk of nitrate and nitrite ingestion. Epidemiological studies have demonstrated the same protection by consuming fruits and vegetables high in vitamin C. Theoretically, any antioxidant should decrease nitrosamine formation in the stomach.

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[a] Registered trademark.

G. Summary

The recommended values for the one-day and ten-day HAs for adults (and all age groups other than the infant) and infants and the AADI are summarized in Table VIII-4.

The current MCL and World Health Organization Standards for nitrate in drinking water are identical, 45 mg/L nitrate ion or 10 mg/L nitrate-nitrogen. No standard exists for nitrite in drinking water but a limit of 1 mg/L nitrite-nitrogen has been recommended.

Risk factors for methemoglobinemia include: (1) drinking water with a high nitrate content, (2) being an infant, pregnant woman or cancer patient, and (3) having a heritable enzyme deficiency affecting ability to reduce methemoglobin.

Risk factors for cancer or mutations induced by N-nitroso compounds include: (1) high intake of nitrates and nitrites, (2) exposure to certain pesticides and industrial air pollutants and (3) use of cigarettes and certain drugs. Vitamin C has been shown to prevent the nitrosation of substrates by nitrite, therefore, reducing the cancer risk of nitrate and nitrite ingestion.

Table VIII-4 Summary of Quantification of Toxicological Effects of Nitrate/Nitrite

	Nitrate-Nitrogen Drinking Water Concentration, mg/L		Nitrite-Nitrogen Drinking Water Concentration, mg/L	
	70-kg Adult[a]	4-kg Infant	70-kg Adult[a]	4-kg Infant
One-Day Health Advisory[b]	111[c]	10[d]	11[c]	1[d]
Ten-Day Health Advisory	111	10[d]	11[c]	1[d]
AADI	--[e]	10[d]	11[e]	1[d]

[a] Values are considered protective for all age groups other than the infant.

[b] No suitable data available to calculate one-day HAs. Values calculated for ten-day HAs are assumed to be protective for one-day exposure.

[c] Reference for NOAEL used in the calculation of one-day and ten-day HAs for the child is Craun et al. (1981).

[d] Reference for NOAEL used in the calculation of one-day and ten-day HAs and the AADI for the infant is Walton (1951).

[e] Not calculated, since the AADI has been derived in order to protect the most sensitive subgroup of the human population (i.e., infants).

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